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CONTENTS

Preliminary Observations on Long Island Sound Sediments. *By* A. W. MCCRONE AND B. F. ELLIS 119
Introductory Remarks. *By* ALBERT S. GORDON 130
Kidney-Bone Marrow Interaction. *By* ALLAN J. ERSLEV 131
Purification and Physiological Effects of Human Urinary Erythropoietin *By* JOHN WINKERT, ALBERT S. GORDON AND ELIZABETH WINKERT 135
Limitations and Design Philosophy in Ultraviolet, Visible, and Near-Infrared Spectrophotometers. *By* JAMES D. MCCALLUM 140
Antibiotics in Experimental Cancer Chemotherapy. *By* MORRIS N. TELLER 158
The Controlled-Climate Chamber for Study of the Effects of Meteorological Changes on Human Diseases. *By* JOSEPH LEE HOLLANDER 167
Design of Climate-Control Chamber. *By* JOHN EVERETTS, JR. 173
Conferences Held 177
New Members 184

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Executive Director: EUNICE THOMAS MINER

SECTION OF GEOLOGICAL SCIENCES

PRELIMINARY OBSERVATIONS ON LONG ISLAND SOUND SEDIMENTS*

A. W. McCrone, B. F. Ellis

Geology Department, New York University, New York, N. Y.

R. Charmatz

Micropaleontology Department, American Museum of Natural History, New York, N. Y.

INTRODUCTION

Area of Study

Geographic setting of Long Island Sound. In this project, bottom sediments were collected from Long Island Sound (FIGURE 1) between New York, N. Y., on the west, and the vicinity of Gull Island, at the eastern end of Long Island. Most of the Sound is less than 100 feet deep. Maximum depths of about 360 feet occur locally northeast of Gull Island. The area of the Sound is about 920 sq. Mi.

Major rivers (Housatonic, Connecticut) which discharge into the Sound at the Connecticut (north) shore, are much larger than those flowing in from Long Island. The drainage basin emptying into the Sound is nearly 20 times larger that the Sound itself. Riley (1956) pointed out that, mainly by way of the Connecticut and Housatonic Rivers, "more than 75% of the runoff enters the relatively open eastern end, where its effect on the Sound as a whole is minimal." Riley also noted that the western end of the Sound is "normally about 5‰ fresher than the eastern end, and there is a seasonal variation of 2 to 3‰." The salinity minimum (25‰) comes with the springtime period of heavy drainage.

Physical oceanography and hydrography of Long Island Sound. Mean tide ranges vary from about 6 feet in the western part of Long Island Sound to about 2½ feet in the eastern part.

Maximum average flood-tide currents are variable between a minimum of approximately 10 cm./sec. in the western part of the Sound and a maximum near 90 cm./sec. at the narrow eastern end near Gull Island. Ebb currents in the same general areas are variable between approximately 15 and 120 cm./sec. Riley (1952) estimated that total inflow and outflow during one tidal cycle is about 8 per cent of the water volume of the Sound below mean low water level. Current velocities may vary due to weather changes (*e.g.* pressure systems), but basic current patterns are

*This paper, illustrated with slides, was presented at a meeting of the Section November 6, 1961. The work described in this paper was supported in part by Research Grant G-13004 from the National Science Foundation, Washington, D. C. New York University provided maintenance of the research ship *Sea Owl*, by means of which all samples were obtained.

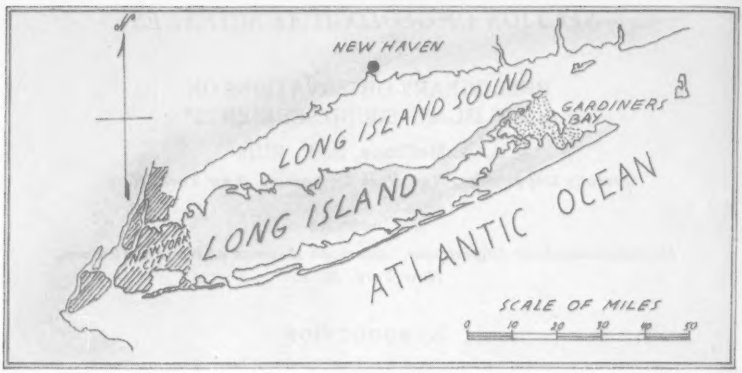

FIGURE 1. Long Island Sound and vicinity.

mainly controlled by bottom topography. Riley (1956) noted that "total freshwater drainage averages about 35% of the volume of the Sound in a year's time." Thus the annual net outflow from the Sound is about one third of its total volume.

Previous Work

During 1946, 1947, and 1949 the Bingham Oceanographic Laboratory, Yale University, New Haven, Conn., conducted hydrographic studies in Long Island Sound (see: Riley *et al.*, 1952). Later, from 1952 to 1954 the Bingham group studied the physical and chemical oceanography, and some of the biology of Long Island Sound (see Riley *et al.*, 1956). Sanders (1956) analyzed some of the bottom sediments upon which certain marine benthic communities live and recognized the definite association between sediment particle size and the presence of certain benthonic genera. He observed that, generally, the "greatest biomasses were found when the sediment contained from 13 to 25% silt and clay."

Shupack (1934), Parker (1948, 1952) and Phleger (1952), described some elements of the Long Island Sound foraminifera.

METHODS AND SCOPE OF STUDY

Sampling and Field Techniques

Approximately 150 cores and 60 spot samples for this study were collected during the summers of 1959, 1960, and 1961, along the 23 traverses shown in FIGURE 2. No samples were taken in near-shore waters shallower than 15 feet because of currents and attendant difficulties of maneuvering the large ship.

Six-foot cores (2-inch diameter) were obtained by means of a free-fall ("percussion") coring tube driven into the bottom sediments by a 500-lb. cylindrical ram. A "wire-basket" sample retaining device was fixed in the coring tube, just above the cutting head, to prevent egress of cored

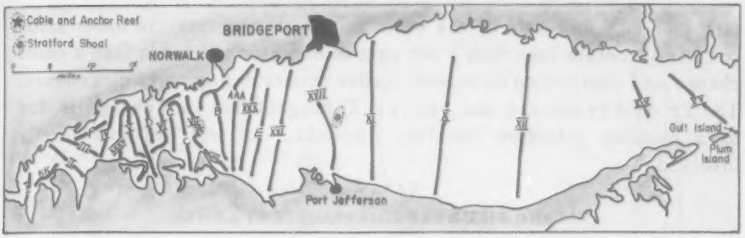

FIGURE 2. Approximate locations of bottom-sampling traverses in Long Island Sound.

sediment. The coring device does not penetrate more than about 1 foot into pure sand.

A few cores were immediately cut open when brought to the surface, and tested for *p*H and Eh, using a Beckman (model g) meter.

Laboratory Studies

Studies of selected samples included: particle size analyses; identification of spores and pollen, foraminifera, and other invertebrate fossils; mineral identifications; estimation of total organic content; and acid-insoluble residue studies. Chemical analyses included observations of: reactive silica; sulfides; calcium and magnesium; basic nitrogen; chloride and sulfate ion; total iron content; and *p*H-Eh relationships in typical sediments.

SEDIMENTS IN LONG ISLAND SOUND

Physical Properties

The majority of sediments collected from the Sound are medium greenish-gray (sandy to clayey) Recent silts. When wet, these muds were quite sticky because of their small content of gelatinous organic colloids and clays. Dried sediments are light gray, firm, and difficult to break, although slightly friable.

Grain size generally increases to sand towards the shore, but a few gravelly lag-concentrate and sandy sediments are present in deep, scoured portions of the floor. Similar coarse clastics occur in shallower, but current-swept areas near submarine topographic highs such as Stratford Shoal and Cable and Anchor Reef (FIGURE 2).

Some of the near-shore sandy silts are shelly. In a few places shells of small pelecypods, with a few gastropods, foraminifers, and oysters, total up to 30 per cent (by weight) of the aggregate.

Black, gelatinous, odorous (H_2S) richly organic mud is common at the top of most silt cores, but is absent in current-scoured areas where sands form the substrate. This suggests that bottom currents in many places are quite gentle, despite the vigor of currents nearer the surface. The

fact that only the tops of the cores are black and that the lower silts commonly contain less than 1 per cent of organics indicates that a color change and destruction of organic matter occurs during early diagenesis. The Eh data (TABLE 1 and FIGURE 3) suggest that this change is due to a complex reduction reaction. Anaerobic bacteria are undoubtedly involved.

TABLE 1

EH AND PH DETERMINATIONS (WET CORES)

Station	Core length (feet)	pH top water	pH bottom water	pH top mud	pH bottom mud	H_2S Qual Det.	Eh top mud (mv).	Eh bottom mud (mv).
Traverse XI (1960)								
4	4	8.30	8.0	7.3	6.7	+	- 75	- 95
5	6	8.35	7.8	7.3	6.9	+	-157	-250
6	6	8.25	7.7	7.6	7.4	+	-390	-500
7	6	8.30	7.8	7.1	7.6	+	- 85	-440
8	6	8.30	8.0	6.9	7.7	+	- 30	- 70
Traverse XVI (1960)								
1	6	7.9	6.5	7.4	7.0	+	-185	-300
2	6	8.1	7.5	7.2	6.8	+	-200	-500
3	6	8.0	7.6	6.8	6.5	+	-200	-600
4	6	8.1	7.1	6.9	6.7	+	-150	-450
5	6	8.2	7.2	7.1	6.9	+	-200	-500
6	6	8.1	7.1	7.0	7.0	+	-150	-400
7	6	8.1	7.4	7.2	6.9	+	-125	-300
8	6	8.1	7.0	6.9	6.7	+	-100	-250
9	6	7.8	6.6	6.8	6.4	+	- 75	-275
Traverse E (1961)								
3	5			7.3	7.7	+	-370	-450
4	5			7.0	7.0	+	-430	-430
7	5			7.1	7.1	+	-270	-370

Natural densities of selected typical greenish-gray silts, taken from 5 to 6 feet below the depositional interface, are commonly about 1.4 to 1.5. Water content of similar samples was about 35 per cent by weight.

Most of the silts from the western half of the Sound are more than six feet thick. Commonly the grain-size distribution in these sediments is remarkably uniform from top to bottom of the cores, but a few sand stringers and fewer clay laminations occur locally. At places where the corer did not penetrate the full six feet, the lowest sediments in the core were sandy or pebbly. The sands stopped the penetration of the core barrel. In some places layers of *Crassostrea* (oyster) shells similarly impeded the coring device.

Some of the sand stringers contain about 40 per cent of foraminifer shells (mostly *Elphidium*) with nearly 60 per cent of subangular and angular clear quartz grains. A few biotite flecks and other mafic minerals

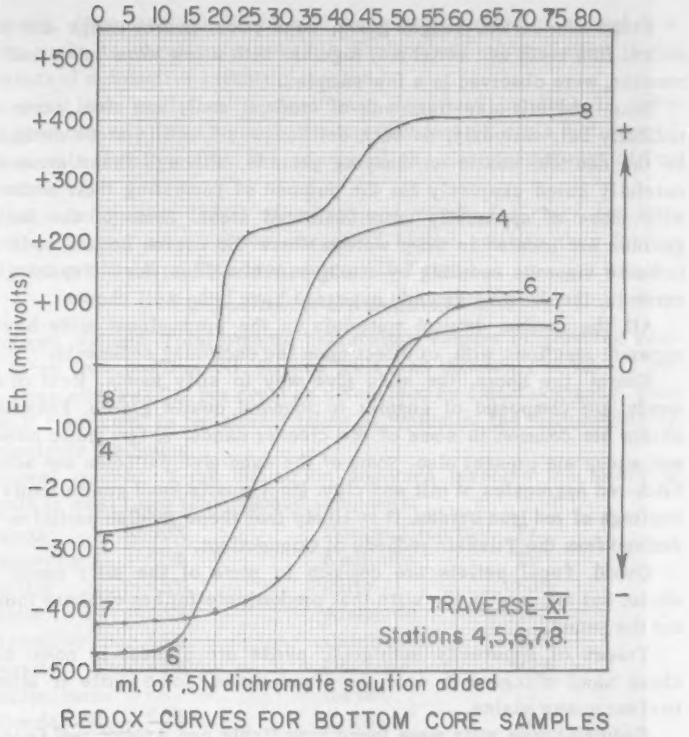

FIGURE 3. Redox curves for sediments at bottom of cores from Traverse XI. Samples were adjusted to *p*H 8.5 (with NaOH) before titration with potassium dichromate. When Eh of zero was reached *p*H was readjusted to 6.0 (using HCl), then titrations were continued.

are also present. The shells themselves are "sand" grains, apparently part of a clean sandy depositional concentrate.

Histograms typical of the common silty sediments show bimodal grain-size distributions wherein coarse and fine silts are conspicuous. Relatively low percentages of clay (commonly less than 12 per cent) are noteworthy because, in the field, the sticky wet sediments give the illusion of being rich in clay.

A few individual sand grains of various sizes, fewer granules, and extremely rare pebbles, occur randomly in the silts. Ice rafting may account for their presence. Likewise, sand-size foraminifer tests and discoid diatoms of very fine sand to medium silt size are randomly sparse throughout the widespread greenish gray silts. Small pelecypod (*e.g. Nucula)* and gastropod (e. *g. Epitonium)* shells of granule and pebble size are rare to common. Ostracodes are extremely rare.

Fragments of tiny twigs, grass, seed pods, insect wings and carapaces, fish teeth and vertebrae, together with a few worm tubes and skin remains, were observed in a few samples.

Sand- and silt-size fragments of cinders, coal, and steel occur sporadically but, curiously, no such detritus was found in areas designated on the nautical charts as dumping grounds, although these areas were carefully cored expressly for the purpose of comparing their sediments with those of apparently uncontaminated areas. Some of the dumping grounds are located in deep waters where the coarse bottom sediments indicate vigorous scouring by strong currents. Thus, for "cleansing" by currents, the dumping grounds appear to have been well chosen.

All the random detrital materials in the predominant silts have no apparent reactions with, or effect upon the enclosing sediments.

Nearer the shore, the silts give way to silty sands. Most of such sands are composed of angular to rounded quartz grains. Foraminifer shells are common in some of the cleaner sands. A few mafic minerals and micas are present also. Some of the sand-size particles are actually brick-red aggregates of silt and clay. Many quartz sand grains retain thin coatings of red iron oxides. It is likely that these reddish particles were derived from the Triassic redbeds of Connecticut.

Ovoid fecal pellets are common in some of the silty sands near shore, but are rare in the silts that predominate farther offshore throughout the Sound.

Traces of apparently authigenic pyrite are present in some of the clean sand stringers in near-shore sediments. Some pyrite is attached to clear quartz grains.

Reddish-brown silts were found near Cable and Anchor reef (FIGURE 2) in 1960 (in 1961 the same area was cored again, but only brownish sands containing a few sand-size red silt aggregates were obtained). These silts differ mineralogically from the greenish-gray silts common throughout the Sound. It was speculated that these red sediments might prove to be of Triassic age, but their spore and pollen content revealed them to be Recent. Nevertheless, it is believed that bedrock is not far below the bottom of this part of the Sound.

Another anomalous occurrence of apparently older sedimentary materials was found during the summer of 1960 in the vicinity of Stratford Shoal (FIGURE 2). A layer of light, slate-gray, tough, sticky clay was encountered beneath 6 inches of Recent sand. This clay sample is mineralogically different and much better indurated than any other fine sediments cored for this project. Its general appearance strongly suggests that it is equivalent to the Gardiners Clay (Late Pleistocene) of Long Island, New York (Weiss, 1954; Perlmutter, 1949). (The same area was cored again in 1961, but only normal Recent sands were encountered).

Radioactivity counts were performed in 1960 on some of the common silts from open parts of the Sound and on silts and sands near harbor

entrances. (Sampling localities will be recored periodically and new counts made, to check upon possible fixation and accumulation in the sediments of radioactive industrial wastes.)

A small but statistically significant downward increase in radioactivity count was noted through the top four feet of a six-foot core of uniform silt lithology from the middle of the Sound northeast of Port Jefferson.

The mean number (33.3) of counts per minute for clays and silts recovered in and near harbors suggested higher activity than for the same size grains taken from open waters (26.7 cpm.)

Composition of the Sediments

Organic content. Total organic hydrocarbon content of selected samples taken from profiles VII, X, and XVII was commonly about 0.1 per cent, (maximum was 0.2 per cent) as determined by chromatography. This value, 0.1 per cent, is tentatively taken as typical of the silty sediments that dominate the Sound.

Mineral content. X-ray diffraction analyses revealed the following minerals in most Long Island Sound sediments:

Quartz	50-90%	Montmorillonite	Tr.
Muscovite	<5%	Kaolinite	Tr.
Biotite	<5%	Illite	<3%
Albite	<15%	Aragonite	<10%
Microcline	<5%	Calcite	<10%
Kyanite	<5%	Dolomite	<2%
Augite, hornblende, etc.	<5%	Garnet	Tr.
		Zircon	Tr.
Chlorite	<5%	Magnetite	Tr.

Recent silts of traverse X (FIGURE 2) (selected as typical of the most common Long Island Sound sediments) contain illite and chlorite, and rare traces of montmorillonite, but no kaolinite. The reddish silts collected near Cable and Anchor Reef (FIGURE 2) contain chlorite and approximately four times more illite than any of the traverse X samples, together with an unnamed mixed-layer mineral composed of an iron chlorite, a calcium-magnesium montmorillonite (having two water layers), and an illite layer. The well-indurated slate-gray clay (Late Pleistocene?) found near Stratford Shoal (FIGURE 2) contains less organics and has finer clays than the recent silts.

Insoluble residue determinations show that soluble carbonates in the common silts average about 8 per cent (values between 5 and 10 per cent are common).

PALEONTOLOGY OF LONG ISLAND SOUND SEDIMENTS

Richard Chamatz and A. W. McCrone

Foraminifera

Foraminiferal content of cores from traverses I, II, III, X, XI, and a

sample from the south side of Gull Island (41° 12″ N lat., 73° 07′ W long.) were studied for this report.

The following foraminifers were recognized: *Textularia torquata, Eggerella advena, Quinqueloculina seminula, Q. seminula jugosa, Q. subrotunda, Trochammina advena, Pseudopolymorphina novangliae, Elphidium clavatum, E. florentinae, E. incertum, E. sagrai, Protelphidium tisburyense, Nonion barleeanum, N. pauciloculum, N. pauciloculum albiumbilicatum, Bolivina pseudoplicata, Ammonia beccarii, Buccella frigida, Hoeglundia elegans, Globigerina sp., Orbulina universa, Planorbulina sp.*

Specimens of *Elphidium* species number more than 50 per cent of the foraminifers in the Long Island Sound sediments. Other genera and species are much less numerous. *Nonion barleeanum* and *Buccella frigida* are rare to common, and the other listed foraminifers rare, but widespread.

Most *Elphidium* species identified from Long Island Sound by earlier authors (e.g. Parker, 1948, 1952; Phleger, 1952) as *Elphidium incertum** *E. bartletti, E. gunteri,* and *E. subarcticum* should be assigned to *clavatum,* a former subspecies which Loeblich and Tappan (1953) and the present authors recognize as specific in rank. Similarly, *Protelphidium tisburyense,* abundant to common in Long Island Sound, was originally described as a species of *Nonion,* but at various times it was assigned to *Elphidium* and *Cribroelphidium.* Our "lumping" of species into *Elphidium clavatum* and *Protelphidium tisburyense* is an attempt to recognize wide ranges of intraspecific varietal forms which reflect changes of form between juveniles and adults, or different forms developed by adaptation to various environments, respectively.

Globigerina and *Orbulina* are the only planktonic forms found in the Long Island Sound samples. *Elphidium sagrai* has not been previously reported from the Sound.

Family Nonionidae

Genus *Elphidium* Montfort

Elphidium clavatum Cushman

Synonymy: *Elphidium incertum* (Williamson) var. *clavatum* Cushman, 1930,, U.S.Nat. Mus., Bull., no. 104, pt. 7, p. 20, pl. 7, fig. 10. *Elphidium clavatum* Cushman – Loeblich and Tappan, 1953, Smithsonian Inst., Misc. Coll., vol. 121, no. 7, Publ. 4105, pp. 98-99, pl. 19, figs. 8-10: – Weiss, 1954, U.S. Geol. Survey, Prof. Paper, no. 254-G, p. 160, pl. 32, figs. 12-14.

Loeblich and Tappan (1953) noted that this species has been frequently misidentified with such forms as *Elphidium bartletti* Cushman, *E. clavatum* Cushman and *E. orbiculare* (Brady). These can be differentiated from typical *incertum* which lacks an umbonal knob. This species is abundant in Long Island Sound.

*Abundant presence of *Elphidium incertum* is also recognized by the present authors.

Protelphidium tisburyense (Butcher)

Synonymy: *Nonion tisburyensis* Butcher, 1948, Cushman Lab. Foram. Res., Contr., vol. 24, pt. 1, p. 21: Ronai, 1955, Cushman Found. Foram. Res., Contr., vol. 6, pt. 4, p. 145, pl. 21, fig. 3: Parker, 1952, Harvard, Mus. Comp. Zool., Bull., vol. 106, pl. 3, figs. 7-8.

The degree of inflation of chambers and rounding of the periphery is highly variable. The genus is differentiated from *Elphidium* Montfort by an apertural pore situated in the middle of the apertural faces.

Corals

Pieces of *Astrangia* were found in a sample taken from western Long Island Sound along traverse VIII (FIGURE 2).

Molluscs

The small pelecypod *Nucula proxima* is common to abundant in Long Island Sound sediments. *Yoldia, Tagelus,* and *Mulina* are less common. Remains of *Crassostrea* are more restricted, and form variably thin patches in silty sands of near-shore areas.

The gastropods *Nassarius, Littorina* and *Epitonium* are rare but widespread in the Long Island Sound silts.

Spores and Pollen

Spores and pollen are common in the Long Island Sound sediments. Almost all specimens found are Recent forms: *Pinus, Tsuga, Betula, Quercus, Abies, Picea, Fagus, Acer, Alnus, Carya,* Compositae, Gramineae; *Osmunda, Sphagnum, Thelypteris, Polypodium, Lycopodium, Selaginella.* A faint alternation of cool- and warm-climate assemblages is detectable in some 6-foot cores. Traces of reworked Paleozoic spores, including *Punctatisporites* (Pennsylvanian) were also recognized.

Diatoms

Flat discoid diatoms (*Coscinodiscus?*) of very fine sand and coarse silt size occur randomly but sparsely throughout all the silty sediments of the Sound.

GEOCHEMICAL OBSERVATIONS ON SELECTED LONG ISLAND SOUND SEDIMENTS

TABLE 1 shows that *p*H's commonly are slightly more acid in bottom muds than in nearly-neutral top muds. Eh values record an increase of reducing conditions towards the bottom of the cores.

Comparison of titration curves 8 and 6 in FIGURE 3 shows similar initial inflection points, indicating maintenance of temporary Eh equilibrium (possibly by Fe++ and S-) before rapid change (oxidation by potassium dichromate) occurred in the Eh of the whole sedimentary aggregate. Organic and clay colloids in the sediments inhibit the rate of aggregate Eh change. Thus curve 5 (FIGURE 3) indicates a comparatively gradual Eh change, and greater total reducing capacity than sedi-

ments of curves 8, 4, or 6, probably because of the relatively greater organic content of the core 5 sediments. Some anaerobic bacteria lower Eh by reducing complex organic substances (proteins, etc.) and releasing H_2S into the sediments. Enzymatic reactions, which temporarily continue after death of organisms, might similarly lower the Eh.

Typical silt sediments contained the following compounds:

$CaCO_3$ 1 to 15%	MgO 0.14 to 1.82%	Organic nitrogen 0.01 to 0.18%
$MgCO_3$ 0.3 to 0.6%	SO_4 = 0.2 to 0.3%	Water 35 to 70%

Interstitial waters contained:

total sulfides 0.4 to 0.7‰	metallic sulfides tr. to 0.2‰
dissolved sulfides 0.3 to 0.5‰	un-ionized H_2S 0.1 to 0.2‰
sodium chloride 25.5 to 26.7‰	reactive silica 4 to 18 ppm

The *p*H-Eh combinations (FIGURE 3) in the silt samples tested showed that, taken as a whole, each wet sedimentary mass possessed reducing capacity. However, this does not preclude oxidizing conditions in microenvironments within the generally reducing sedimentary aggregate.

Acknowledgment

Special thanks to Stephen Kaplin for doing the geochemical analyses.

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SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

INTRODUCTORY REMARKS*

Albert S. Gordon

Department of Biology, Graduate School of Arts and Science, New York University, New York, N. Y.

In 1906, Carnot and Déflandre[1] proposed the concept of a circulating erythropoietic stimulating principle which they termed "hemopoietine." Since that time, many investigators have attempted to provide evidence for this contention.[2] However, most of the results obtained before 1950 were of questionable significance. It would seem that faith, rather than unequivocal demonstration, kept this concept alive, for it appeared that certain aspects of the regulation of red cell formation could best be explained on a humoral basis.[3]

It is only in the last few years that convincing evidence for the existence of an erythropoietic stimulating factor (ESF or erythropoietin) in the body fluids of animals subjected to various forms of hypoxia and of anemic human subjects has been obtained. This can be attributed in part to the development of more sensitive assay procedures[4] and to the administration of considerably larger quantities of plasma or serum[5] that represent the only reliable sources of the factor.

Despite the resurgence of interest and work in this field, some areas still remain incompletely resolved. These relate to the site(s) of production, locus of action, metabolism, and chemistry of the ESF.

Several aspects of these pertinent problems will be discussed in the following papers.

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*These remarks introduced three papers presented at a meeting of the Section held on November 13, 1961. Two of these papers follow. The third paper, entitled "Regulation of Erythropoiesis," by Frederick Stohlman, Jr. of the Public Health Service, Bethesda, Md., will appear in the January issue of the *Transactions*.

KIDNEY-BONE MARROW INTERACTION*

Allan J. Erslev

Cardeza Foundation, Jefferson Medical College, Philadelphia, Pa.

The red cell mass is a large organ designed primarily to bring oxygen from the lungs to the tissues. Its size is determined by the demands for oxygen in the tissues and by the need to conform to the functional capacities of other organs. It cannot be too small since this would result in tissue hypoxia or would demand excessive compensatory efforts by the cardiovascular and pulmonary systems. On the other hand it cannot be too large since the associated increase in blood volume and viscosity and decrease in plasma volume would infringe on cardiovascular and renal functions. An optimal homeostatic balance appears to be reached in mammals at sea level when the red cell mass is about 30 ml/kg. body weight.

This balance is upset if the supply of oxygen to the tissues does not meet the demand either because of an increase in the rate of metabolism or by a failure to transfer enough oxygen from the air to the tissues. The resulting tissue hypoxia is temporarily corrected by an increase in the pulmonary and cardiovascular functions. However, if the oxygen deficiency is sustained the immediate emergency defense is replaced by a slower but more economical expansion of the oxygen-carrying capacity of blood. If the oxygen supply should happen to exceed the tissue demand, the oxygen-carrying capacity will contract slowly until a new equilibrium has been reached. The attainment and the maintenance of an optimal oxygen-carrying capacity is achieved by the bone marrow.

Like other organs with homeostatic functions, the bone marrow must be controlled by a feedback system. The system that appears most appealing is one in which the tissue tension of oxygen controls the rate of red cell production and the rate of red cell production reciprocates by adjusting the tissue tension of oxygen[1].

It has been proposed that this feedback circuit goes through the following steps (FIGURE 1).

In the first step stem cells from a pool in the bone marrow differentiate to early nucleated red blood cells. These cells proceed through a number of divisions to mature to reticulocytes and adult red blood cells. The rate of red cell production and the rate of red cell destruction determine the size of the red cell mass. Since the life span of the red cells is not a physiologic variable, the control of the size of the red cell mass is determined by the rate of red cell production. In the next step the size of the red cell mass controls the concentration of hemoglobin in circulating blood. Under certain pathologic conditions and in extreme anemias and polycythemias plasma volume may play a contributing role, but under

*This paper, illustrated with slides, was the first of three papers presented at a meeting of the Section on November 13, 1961.

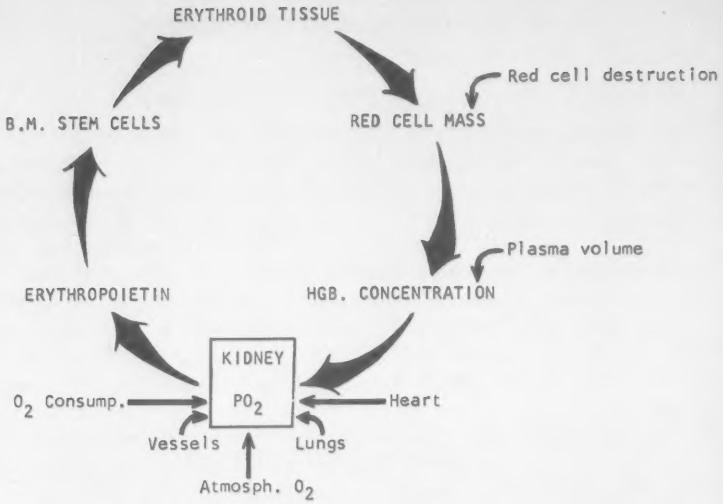

FIGURE 1

most conditions the hemoglobin concentration is directly proportional to the red cell mass. The hemoglobin concentration, which stands for the oxygen carrying capacity of a certain volume of circulating blood, will, in cooperation with other organs, determine the amount of oxygen delivered to the tissues. Because of the extensive mutual compensation among the organs at this step, it is often very difficult to relate a specific hemoglobin concentration to the tissue tension of oxygen. Furthermore, since the tissues have varying rates of oxidative metabolism, and varying blood supply they must also have varying "tissue tension of oxygen." Recent studies have suggested that it may not be the over-all tissue tension of oxygen but the tissue tension in one area, the kidney, which is of importance. The specific tension of oxygen here is thought to lead to the release of an appropriate amount of erythtopoietic factor or erythropoietin into the circulation. In the final step this hormone will reach the bone marrow where it finishes the hypothetical feed back circuit by controlling the rate by which stem cells are differentiated to prcnormoblasts. There may be additional actions of erythropoietin on the maturation or release of early red cells. However, the principal action that leads to sustained changes in the red cell production is the action on the stem cells.

The great bulk of clinical and experimental studies on anemias and polycythemias is compatible with the existance of such an interaction between the kidney and the bone marrow. Admittedly there are observations that do not fit readily into this scheme but these observations appear to demand minor modifications of the hypothesis rather than a complete revision.

The beauty of this hypothesis is that the kidney, which already is known to influence or control blood pressure and plasma volume is also given the control of the red cell volume. Up until 1957, the kidney was thought to influence the bone marrow merely by providing a metabolic environment conducive for red cell production. In that year, however, Jacobson and his co-workers reported that nephrectomized rats fail to generate significant amounts of erythropoietin when exposed to anemic or hypoxic anoxia and they proposed that erythropoietin is produced by the kidneys. This important observation has been amply confirmed in rabbits[4], rats[5] and dogs.[6] At first it was questioned whether the lack of generation actually was caused by the removal of renal tissue or was caused by the associated uremia. This question was especially pertinent for rabbits in whom uremia induced by bilateral ureterligation, suppresses the generation of erythropoietin as well as erythroid activity.[7] However, in rats and dogs the suppressive effect of uremia alone does not appear to be as severe, and in these species the difference between the production of erythropoietin in nephrectomized and uriterligated animals is very convincing. It is possible that the lack of erythropoietin production in ureterligated rabbits is due to the fact that renal tissue in the rabbit is more easily destroyed by the effect of an increase in urinary hydostatic pressure than is renal tissue in rats and dogs.

Recent studies have shown that extracts from renal tissue contain small amounts of erythropoietin.[8] Extracts from renal tissue obtained from anemic animals appear to be slightly more active than extracts obtained from normal animals, but the differences are small and not quite convincing.[9]

According to the present hypothesis, the tissue tension of oxygen in the renal tissue should control the production or release of erythropoietin. This tension is influenced by the special vascular architecture of the kidneys and may not always reflect the tissue tension of oxygen in other organs. Better understanding of this relationship may lead to the answer of a number of troublesome questions: (1) Why does not the compensatory increase in cardiovascular function seen in anemias abolish tissue hypoxia and erythropoietin generation? (2) Why does not polycythemia, which is associated with an increase in viscosity and a decrease in blood flow, lead to tissue hypoxia and erythropoietin generation? (3) Why do patients with compensatory hemolytic anemia have an accelerated rate of red cell production despite a normal hemoglobin concentration?

Although the kidneys appear to be the major sites for the production of erythropoietin, there must also be other sites of production. Nephrectomized rabbits kept alive by peritoneal dialysis continue to generate small amounts of erythropoietin and continue to produce red blood cells in their bone marrow.[7,10] In man, Rees and his co-workers[11] have observed definite erythroid activity in 6 patients despite the complete lack of functional kidney tissue (2 were nephrectomized and 4 had combined cor-

tical and medullary necrosis). This residual activity appears to reflect a low but steady production of erythropoietin since a complete suppression of its production (transfusion polycythemia, high atmospheric pO_2) has been shown to reduce erythroid activity to zero.

The location of the extrarenal site (or sites) of production is not known It is possible that certain cells will generate erythropoietin when forced be anoxia to use anaerobic metabolic pathways. These cells could be of the type found in some tumors of the kidney, liver or cerebellum, which have been found to contain erythropoietin and induce polycythemia. If this is so it may be assumed that these cells are present in the kidney either in large numbers or in sites where the vascular architecture of the kidney render them very sensitive to changes in the body's supply and demand for oxygen.

In conclusion it appears that the major control of red cell production is accomplished by a feedback mechanism that runs between the bone marrow and the kidney, mediated in one direction by hemoglobin-bound oxygen and in the other by erythropoietin. However, a number of observations do not fit readily into this feed back circuit suggesting the existence of a more complex pathway or of additional controlling devices.

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PURIFICATION AND PHYSIOLOGICAL EFFECTS OF HUMAN URINARY ERYTHROPOIETIN*

John Winkert,† Albert S. Gordon, Elizabeth Winkert

Department of Physiology and Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals and Department of Biology, New York University, New York, N. Y.

The repeated demonstration of the existence of erythropoietic stimulating activity in the plasma and urine of a variety of animal species[1-5] and the newer, more refined evidence indicating that the potency of these fluids increases with the severity of anemia in the donors[6-8] has led to a general acceptance of the original proposal of Carnot and Déflandre,[9] that erythropoiesis is regulated by a humoral agent. Carnot and Déflandre termed this substance "hémopoiétine," but it has more recently been referred to as the erythropoietic stimulating factor (ESF)[10] or erythropoietin.[11]

Reticulocyte Release

If apart from the necessary metabolic intermediates, minerals, vitamin coenzymes, and known hormones that play a supportive role in erythropoiesis, ESF represents a prime regulator of red cell regeneration after blood loss, then one would expect that the proper administration of ESF should evoke a reticulocyte response pattern similar to that produced by bleeding. The response to a single bleeding of 20 per cent of the rat's blood volume is illustrated in FIGURE 1. It may be observed that there is an early slow discharge of reticulocytes into the circulation followed on the second day after the bleeding by an increasing rate of appearance of reticulocytes in the peripheral blood. Our first attempt to reproduce this curve by injecting human urinary ESF into rats was unsuccessful (TABLE 1). We had noted that all the additional reticulocytes detected in the circulation after the daily subcutaneous administration of 14 HR units[7] of human urinary ESF to rats were discharged within two hours after each injection. Two more urine samples produced similar responses. A fourth sample, however, which had pronounced erythropoietic potency, lacked this ability to induce an abrupt reticulocyte discharge (FIGURE 1).

Although it is not yet clear whether the difference between the results presented in FIGURE 1 and in TABLE 1 is a result of the presence of accelerators or inhibitors of reticulocyte release in these preparations, it may be concluded that reticulocyte releases at the rates indicated in the first table are not an essential part of the erythropoietic action of ESF. It seems likely that the exact mode of action of ESF will not be revealed until chemically pure ESF becomes available.

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†Postdoctoral Fellow of the Helen Hay Whitney Foundation, New York, N. Y.

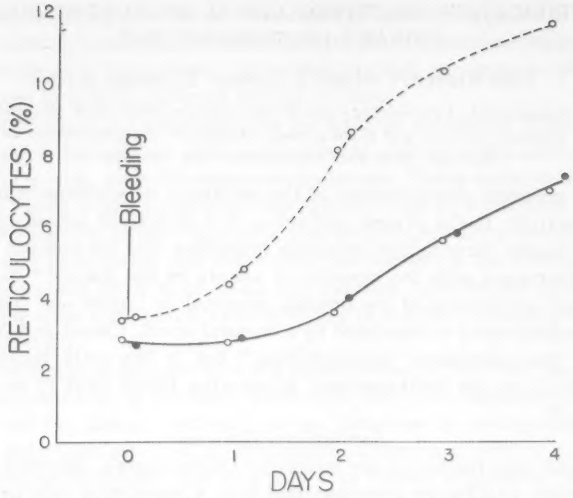

FIGURE 1. Time course of the reticulocyte responses to bleeding and injection of ESF. The broken line is drawn through the mean reticulocyte percentages of 6 rats after a single bleeding. The solid line is drawn through the mean reticulocyte percentages of 12 rats treated with 14 HR units of human urinary ESF per day. Open circles: mean values 2 hours before injections. Closed circles: mean values 2 hours after injections.

TABLE 1

RETICULOCYTE RESPONSE TO HUMAN URINARY ESF

Day	Time after Injection (hours)	Percent Reticulocytes Mean ± S.E.	Increment in Percent Reticulocytes Mean ± S.E.		Value of P
			Treated rats	Control rats	
1	-2	3.1±0.5			
1	2	3.7±0.4	0.6±0.2	0.0±0.3	NS
2	22	4.0±0.8	0.3±0.4	0.1±0.5	NS
2	2	4.8±0.6	0.6±0.3	-0.2±0.2	<0.05
3	22	4.6±0.3	-0.2±0.5	-0.1±0.3	NS
3	2	6.4±1.2	1.8±0.6	0.1±0.3	<0.05
4	22	6.2±0.7	-0.2±0.6	0.2±0.5	NS
4	2	8.6±1.1	2.4±0.5	0.2±0.3	<0.05
5	22	8.8±1.3	0.2±0.3	-0.3±0.3	NS

*S.E. is the standard error of the mean. Each mean value represents the average reticulocyte percentage of seven rats.

Purification of Human Urinary ESF

Urine from thalassemia patients at hemoglobin levels of 4.0 g. per cent or less are screened for ESF activity by the method of Winkert and Gordon.[7] Only those samples possessing an activity greater than 14 HR units[7] are accepted for processing. The frozen urine is thawed and filtered at 5° C. through Whatman No. 1 paper. Alcohol is added to the filtrate which is stirred constantly with the aid of a magnetic stirrer. A precipitate containing the ESF forms in a few minutes, but is usually spun down 5 to 12 hours later. The alcohol is decanted and drained off by inverting the centrifuge tubes. The precipitate is then dissolved in an amount of sterile 0.16 *M* NaCl-0.25 *M* Na_2HPO_4-*p*H 7.4 solution equivalent to 33 to 40 per cent of the original urine volume. Although in a test run the yield of ESF has been the same regardless of whether the precipitation was performed at -10° C., 5° C., or 21° C., we have generally carried out the precipitation at -10° C. Methyl, ethyl, or propyl alcohols have been used for the precipitation. At 80 per cent v/v, methanol afforded the highest yield (62.5 per cent). This was surprising since the dielectric constant of the medium is not lowered as much by methanol as it is by ethanol. Ethanol, at 63 per cent, v/v concentration, however, with about the same effect on the dielectric constant as 80 per cent methanol did precipitate an equivalent amount of ESF. Routinely, however, 80 per cent ethanol has been used for the precipitation with an average yield of about 39 per cent.

The sterile buffered saline solution of the alcoholic precipitate is then dialyzed 4 days in Vis-King 20-32 tubing at 5° C. For the first 2 days dialysis is performed against 10 l. of 0.16 *M* NaCl-0.25 *M* Na_2HPO_4 -*p*H 7.4. For the second 2 days, dialysis is carried out against 10 l. of 0.005 *M* Na_2HPO_4-*p*H 7.4 which is renewed each day. Between the dialysis against the high and the low salt solutions, an equilibration with 0.5 per cent suspension of DEAE-cellulose has removed a proportionately larger amount of pigment absorbing at 400 mμ than protein absorbing at 280 mμ, but this step has not increased the specific biological activity of the final product.

After dialysis, the dialysand is equilibrated with DEAE-cellulose either in batches or in a column. The ESF adsorbed onto the DEAE-cellulose can be eluted at *p*H 7.4 with 0.005 *M* Na_2HPO_4 and a continuous or discontinuous salt gradient. With a continuous salt gradient and elution from a column as many as 5 active peaks have been eluted with the most potent ones eluting below 0.12 *M* NaCl-0.005 *M* Na_2HPO_4. After lowering the salt concentration of the effluent with the most potent peak by dialysis or by dilution and rechromatography on DEAE-cellulose, a single peak is eluted that generally contains the highest specific biological activity in the center and the lowest on the sides (FIGURE 2), indicating that ESF is likely to be the principal component in the peak. That other components are present is evident from the changes in the

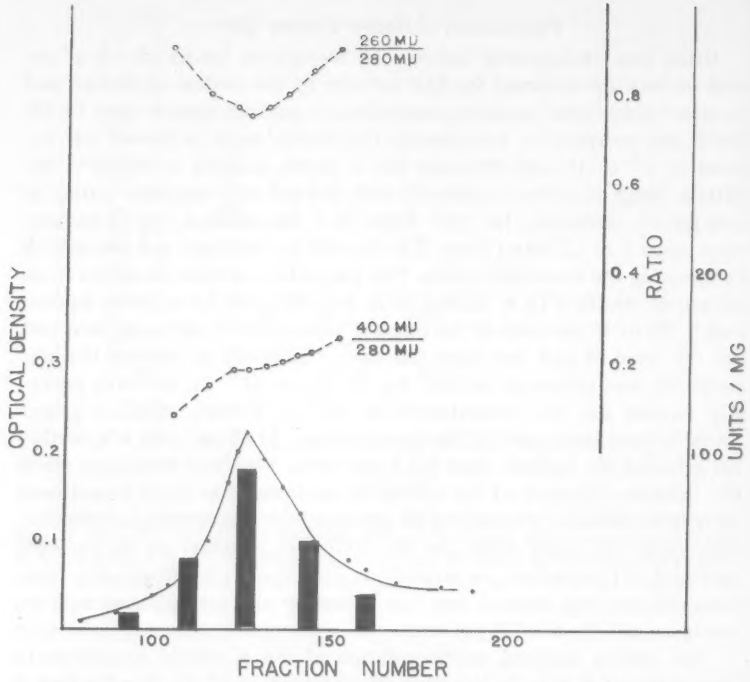

FIGURE 2. Elution pattern of a methanol precipitated preparation of human urinary ESF after rechromatography on DEAE-cellulose. The potencies are given in terms of cobalt units determined by the method described by White *et al.*[12]

optical density ratios from one end of the peak to the other. By cutting the peaks in the first chromatographic run closer to the summit it has been possible to obtain a peak in the rechromatography step that has constant optical density ratios from one end to the other. The maximum sedimentation coefficient for this material is approximately 1 Svedberg unit, which is far less than that of the 40,000 molecular weight material obtained from sheep.[12]

It is possible that a phospholipidlike substance may be binding to human urinary ESF and is therefore responsible for the apparent lowering of the density of the factor. Since it is generally agreed that ESF is an alpha globulin,[12-14] it is not surprising that many substances may be found associated with it. A partial list of the possible contaminants includes FSH,[15] transcortin,[16] thyroxine,[17] and vitamin B_{12}.[18] In addition, we have noted that Armour lot number K103-124 erythropoietin, but not human urinary ESF, has the capacity to expand amphibian melanophores[19] whereas Armour lot number D-216-155CAB beta-MSH was found not to be erythropoietic in the assay described by White *et al.*[12]

We hope that the newer techniques of gel and immunoelectrophoresis as well as further studies employing biological assays for a wider variety of trace contaminants will help in the resolution of a single ESF component from this truly microheterogeneous alpha globulin material.

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DIVISION OF INSTRUMENTATION

LIMITATIONS AND DESIGN PHILOSOPHY IN ULTRAVIOLET, VISIBLE, AND NEAR-INFRARED SPECTROPHOTOMETERS*

James D. McCallum
Beckman Instruments, Inc., Mountainside, N. J.

Introduction

The field of instrumentation is such a rapidly growing one that at times, one finds himself in the midst of a bewildering array of instruments designed to do an equally large number of jobs. The selection of an instrument to do a particular job may be based on a variety of factors; price, performance compared to its competitors, size, reputation, and even at times the looks of an instrument may influence a prospective user one way or the other. Whatever may be his reasons for choosing an instrument, what he actually gets is the combined result of a number of decisions that the manufacturer has had to make in designing and building it.

The purpose of this paper is to discuss some of the limitations and compromises with which any instrument manufacturer is faced in designing an instrument, how these factors are optimized, and how these optimized decisions influence the use of the end product.

I shall confine my remarks to ultraviolet, visible, and near-infrared instruments, and I shall show how these various decisions have been applied to a newly designed double beam ultraviolet spectrophotometer.

General Considerations

First let us consider that any spectrophotometer, no matter how complicated it may appear externally, is composed of the same five basic components shown schematically in FIGURE 1: (1) a radiant energy source; (2) a detector; (3) a monochromator; (4) a sample compartment; and (5) a readout mechanism. FIGURE 2 shows one typical way in which these might be arranged.

Sources

If we start our discussion with the first link in the chain, the radiant energy source, immediately we encounter some problems. First, no single source is available that will cover the entire ultraviolet, visible, and near-infrared regions. Second, sources are quite nonlinear in their output. Consequently, at some wave lengths we have energy to spare, while at others we are energy-limited. There are several ways to cope with this situation, as we shall see later.

*This paper, illustrated with slides, was presented at a meeting of the Division on November 14, 1961.

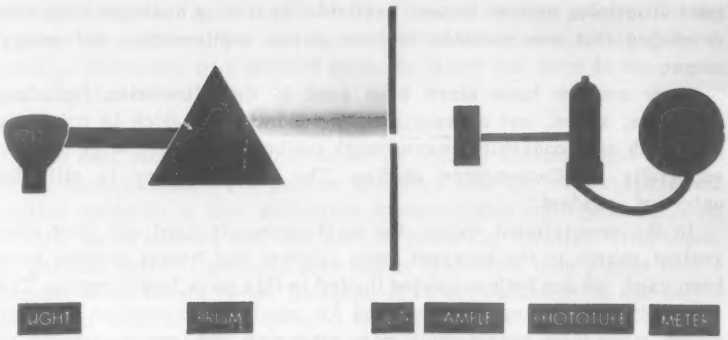

FIGURE 1. Five basic spectrophotometer components

The most commonly used sources for ultraviolet, visible, and near-infrared spectrophotometry are the hydrogen discharge lamp for the ultraviolet, and the tungsten lamp for the visible and near infrared. The ordinary hydrogen lamp may be limited on the lower end (below about 200 mμ) by the absorption of its silica envelope. By using special window material, this range can be extended to 160 mμ. On the upper end its useful range stops at 360 mμ. Beyond this, the spectrum of hydrogen changes from a continuum to the Balmer series of discrete emission lines. The tungsten lamp is also limited at both ends. The normal glass envelope absorbs ultraviolet radiation below 300 mμ and near-infrared energy above 3500 mμ. Therefore, the tungsten lamp is limited to the visible and near-infrared regions. Its peak energy point is about 1000 to 1500 mμ at normal operating temperature.

Source problems were among the first limitations encountered in the design of early spectrophotometers.[1] At the time, convenient UV sources were not available. The first compromise was to use tungsten light sources and operate the instrument in the visible, but to design the instrument so that the conversion could be made as easy as possible when

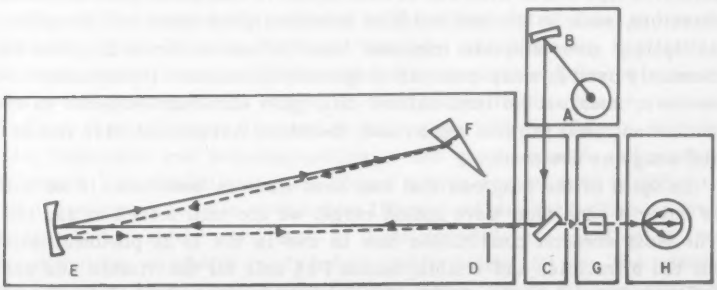

FIGURE 2. DU optical diagram

good ultraviolet sources became available. In time, a hydrogen lamp was developed that was suitable in size, power requirements, and energy output.

Many sources have since been used in the ultraviolet, including deuterium, xenon, and mercury lamps, the latter of which is commonly used both as a convenient wave length calibrator and an energy source, especially for fluorescence studies. The hydrogen lamp is still the universal standard.

In the near-infrared region, the most commonly used and most convenient source is the tungsten lamp. Globars and Nernst glowers have been used, but are both somewhat limited in this wave length region. The necessity for cooling a Globar and for regulating current to a Nernst glower make them considerably more expensive and less convenient as sources. Arc lamps, such as the carbon arc, may also be used if more intense radiation is needed.

The last source I shall mention is the flame, in which the sample itself becomes the source and the monochromator serves to analyze the emission or absorption spectrum thus produced.

Detectors

Detectors also represent an area where manufacturers must bow to present technology in making their choices. Detector studies have progressed much over the years, but we are still somewhat limited to a relatively few types.[2] These may be divided roughly into three classes: thermal, photoemissive, and photoconductive. Thermal detectors, such as the thermocouple, possess a very wide frequency response range, but are limited somewhat in signal-to-noise ratio by their sensitivity to room radiation. Thermocouples are basically heat detectors and are bathed in an environment of heat under the usual conditions. Pneumatic detectors, such as the Golay cell or the Luft type pneumatic detector, have a very wide range and, in the case of the Luft cell, may be made quite selective by proper choice of the filling gas. Detectors of this selective type are in wide use in nondispersive analyzers. Photoemissive detectors, such as the red and blue sensitive phototubes and the photomultiplier, give adequate response from 160 mμ to about 1 μ and are commonly used in many commercial spectrophotometers. Photoconductive devices, such as the lead sulfide cell, give excellent response in the visible and near infrared region and, therefore, have found wide commercial usage as detectors.

In spite of the progress that has been made in detectors, if we wish to cover a very wide wave length range, we are still forced to use two. The most common combination now in use is the 1P28 photomultiplier for the ultraviolet and visible, and a PbS cell for the visible and near infrared. The necessity of changing detector in the middle of the range is a limitation that instrument manufacturers must face much the same as the

source limitation. As a result of this, it is a common practice for designers to provide either an interchangeable detector mounting plate, a sliding positioner, or a pivoted mirror to direct the beam to the proper sensing element.

Monochromators

The next section of the instrument on which a decision must be made is the monochromator. The primary questions here are what systems and optical materials to use. Reflective systems using gratings and prisms, or both, are in common use, and each of these has peculiar advantages. In general, suitable gratings give higher dispersion than prisms over a broader wave length range. However, it is not sufficient to use only a grating to obtain a high dispersion spectrum. Gratings have the inherent problem of stray radiation from overlapping orders. This can be combated by using filters or an order sorting prism.

Prisms, while they do not have stray light problems to the same degree as gratings, do have the property of nonlinear dispersion. The spectral band width of the emergent beam will therefore vary with wave length unless the slit opening is also varied with wave length. In such a monochromator, steps must be taken to vary the slit width to produce essentially a constant spectral band width, whereas in a pure grating monochromator a fixed slit or constant band pass may be used when working in a single order.

For work in the ultraviolet, and especially the far ultraviolet, prisms provide excellent dispersion, and good resolution may be achieved in the ultraviolet and far ultraviolet using a single quartz prism. It is actually possible to achieve resolution of the order of 0.5 mμ at 180 mμ with this type of system.[3]

By combining the two in a foreprism-grating double monochromator arrangement, one may capitalize on the desirable features of both. In the ultraviolet and far ultraviolet the prism provides high dispersion; in the visible and near infrared, the grating provides most of the dispersion; and throughout the whole region, the prism acts as an order sorter to eliminate stray radiation.

The most common prism material for the ultraviolet visible, and near-infrared regions is natural quartz. The only real limitation it poses is its own purity. FIGURE 3 illustrates the comparison between natural and synthetic quartz with respect to their transmittance characteristics. Ordinary natural quartz is good down to about 200 to 205 mμ. Beyond this, impurities and inhomogeneities in the quartz absorb and scatter radiation. With careful selection, natural quartz may be used to 185 mμ, but if the objective is to work below 185 mμ, either fused silica or synthetic quartz must be used. Both of these materials are excellent in the far ultraviolet, synthetic quartz being usable below 160 mμ. The principal limitation here is in the near infrared region. Fused silica is limited to about 2650 mμ because of entrained water which gives rise to strong

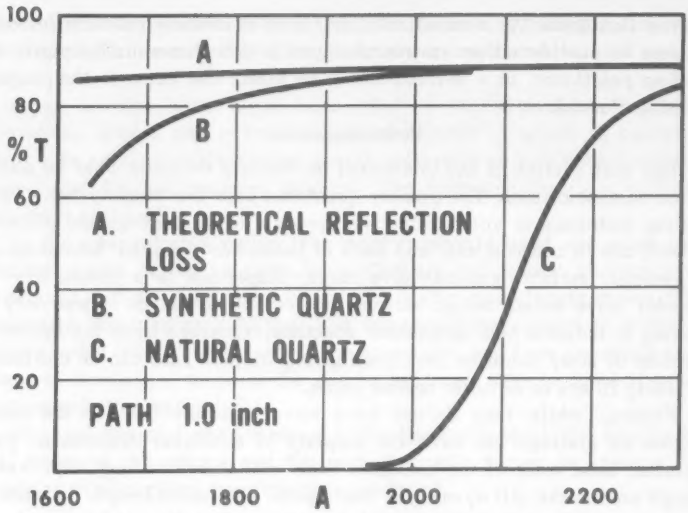

FIGURE 3. Transmittance characteristics of quartz

hydroxyl absorption bands in the near infrared. Synthetic quartz does not have this trapped water and consequently may be used as far as 3500 mμ.

If the instrument is to cover only the visible region, borosilicate glass gives acceptable transmission from about 325 mμ through the visible region and partway into the near infrared. FIGURE 4 illustrates

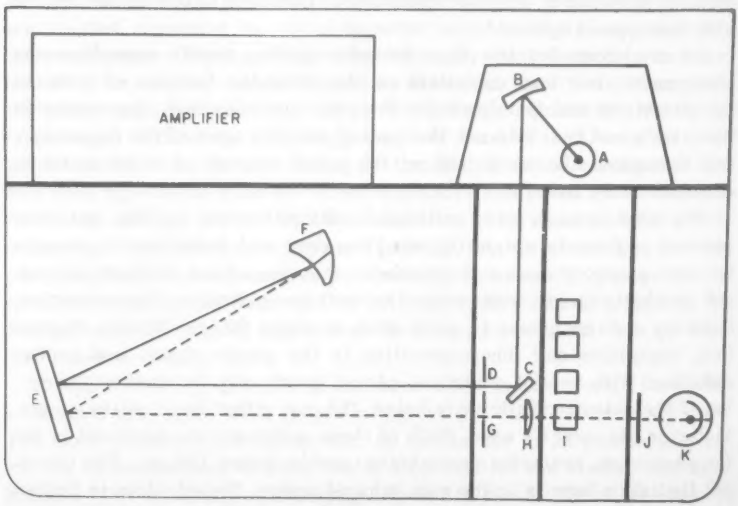

FIGURE 4. Model B optical diagram

the optical diagram of a glass prism spectrophotometer[4] and serves to show how a particular technological development may affect the design of an instrument. The prism in this instrument is a Fery prism, which, unlike a flat prism, acts as its own focusing lens. However, since the focal length of a lens changes with wave length, a special mount is needed that will move the prism laterally as well as turn it on its axis so that it remains in proper focus at the exit silt.

Whether we choose prisms or gratings as our optical material, we still have another fundamental problem to cope with: the wave length dependent variation in source output, detector sensitivity, prism dispersion (or grating efficiency), and background absorption. These combine to give us a system in which the over-all energy level varies with wave length.

If we are to maintain a reasonably constant response, we must compensate for this variation. This may be done in one of two ways – optically or electronically. In a manually operated unit such as the DU Spectrophotometer, we can do it optically by setting the wave length and sensitivity control and adjusting the slit opening to give a 100 per cent T reading. Or we can set the wave length and slit opening and vary the sensitivity setting to give a 100 per cent T reading. There are compromises to be made in both these modes of operation. In the former, we compromise on resolution to minimize noise level. In the latter, we compromise on noise level to gain resolution or sensitivity. The mode of operation chosen depends on the requirements of the particular analysis we are making.

In an automatic recording instrument, optical compensation may be accomplished by varying the slits as a function of wave length or background energy, using either a cam programmed slit system or a slit servo system. Electrical compensation may be accomplished by varying the amplifier gain and time constant or by varying the scanning speed as a function of background energy. In a ratio-recording quartz prism spectrophotometer, reference beam energy is maintained constant by taking the signal from the reference beam and feeding it through a reference signal amplifier to a servomechanism that continually adjusts the slit opening to maintain a constant reference energy level. In double beam operation, as source intensity or detector response varies, the slit follows the variation.

In differential analysis where the reference material absorbs strongly, or while operating in the water region of the near-infrared, the slit servo systems acts to provide a constant reference energy. In a pure grating system using linear dispersion or constant bandpass, the slit opening is fixed. System energy fluctuations caused by source and detector nonlinearity and background absorption must therefore be compensated electrically by automatically controlling the gain or the sensitivity of the detector.

The Sample Compartment

The sample compartment is the point at which we decide whether we shall have a single beam or a double beam instrument, and in the case of a double beam system it is usually the place where beam switching or light modulation is performed. It is also the point at which accessory versatility is usually incorporated. Here provisions may be made for special cell arrangements such as temperature regulated cells, flow cells, reflectance attachments or special external optics. The physical layout of the instrument to a great extent determines just how much versatility can be built in, and the prior choice of one has much to do with fixing the other.

Readout Mechanism

The readout section of the instrument is the one where the greatest amount of variation exists among manufacturers. Here the choice is made among null electrical balance, ratioing, direct reading, optical null, whether it is to be a recording instrument, and if it records, whether it uses a strip chart, a flat bed, or a drum recorder. On the basis of existing instruments, one can generalize that high performance and high speed are incompatible. To use the DU Spectrophotometer as an example, we have a single beam, null balance instrument possessing very high photometric accuracy. Readings are taken point by point, and at each point the operator must make several manual adjustments. The point here is that the readings are static readings and are inherently more accurate than dynamic readings on the same instrument, since the manual instrument is at statistical equilibrium when the reading is taken. In addition, the null electrical balance method is probably the most accurate means of making a transmittance measurement because accuracy depends only on slidewire linearity, and linearities of better than 0.1 per cent are quite readily accomplished. The necessity for manual adjustment of instrument settings, however, places a limitation on the speed with which the measurement may be made.

In certain routine applications, it may be possible to sacrifice some of the extreme accuracy of a manual null balance instrument for the increased speed of a manual direct reading instrument with a meter readout. Incorporation of an adapter to eliminate the need for transferring solutions from test tube to absorption cell is an aid in gaining rapidity of operation and increased accuracy. An example of this is shown in FIGURE 5. The limitation that this places on the manufacturer is twofold. The first is meter accuracy. Meters are limited to accuracies of about ± 1 per cent. In applications where photometric accuracy requirements of this order are permissible, however, meter readout is often adequate. The second limitation is amplifier stability, or the ability to make successive readings over an extended period without frequent adjustment of the controls. Feedback stabilized amplifiers provide this stability and are commonly used.

The next obvious step in the "performance versus convenience" scale is a recording instrument with the same performance characteristics as the nonrecording instrument on which it is based. The comparison

FIGURE 5. Model B test tube adapter

chosen here is the DU and DK Spectrophotometers. The increased complexity of the DK is a result of a decision to optimize the system to obtain speed and convenience and still maintain the necessary degree of accuracy for analytical measurements.

If we could transfer all the performance characteristics of a nonrecording instrument to a recording instrument without loss, it would indeed be an ideal situation. In practice, it is often difficult to buy back speed and convenience without loss in some area of performance. Necessary changes in the layout of the various components to get the degree of convenience and versatility we want can cause significant differences in the results obtained on the two instruments. This is one of the major reasons why discrepancies exist in measurements on the same material made with instruments of different design. These factors do not only manifest themselves among instruments made by different manufacturers, but will also appear when comparing measurements on the same sample using instruments of different design made by the same manufacturer.

As an example of this, let us consider one of the basic differences between the DU and the DK. In order to incorporate the 15-cycle beam switching into the DK and to permit the use of longer absorption cells, a longer sample compartment was required. The difference here is that in the DU the distance from the sample to the phototube is about 1 inch and in the DK is about 10 inches. Consequently, suspended particles in the sample or cell imperfections that cause scattering will cause more energy loss in one instrument than in the other, resulting in a higher apparent absorbance observed with the DK. In turbidity measurements, however, the longer sample-to-phototube distance may be turned to advantage, since it tends to provide a more sensitive measurement because of the greater scattering loss caused by the extra distance.

The Model DB

Let us now use these principles to describe a recent development in spectrophotometers[3] and illustrate how some of the factors discussed are incorporated into its design, with special emphasis on the interrelationship between the various individual design features and the limitations introduced due to their selection. The instrument is the Model DB Spectrophotometer (FIGURE 6), which is a quartz prism ultraviolet-visible spectrophotometer for the range 220 to 700 mμ. Limitation of the DB to this wave length range is due to several important factors, the first of which is the class of applications toward which the DB is oriented. By and large, these are routine measurements in the visible and ultraviolet that do not demand either the resolution or the extreme photometric accuracy of a manual, null balance instrument, but do require accurate measurements at discrete wave lengths with speed and simplicity.

Second is the spectral response of commercially available detectors and the advisability of only using one detector, if at all possible. The

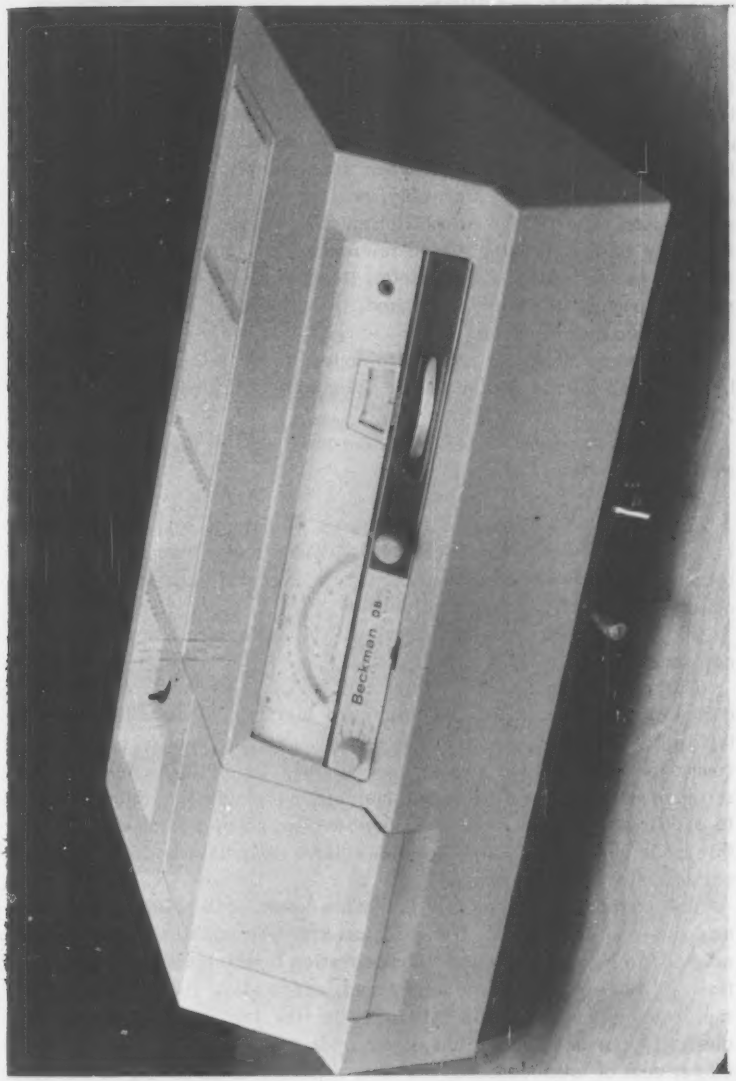

FIGURE 6. Model DB spectrophotometer

1P28 photomultiplier offers an adequate response throughout most of the 200 to 700 mμ range, although at the extreme red end of the range it is somewhat limited in sensitivity.

The general component layout of the DB is shown in FIGURE 7. The optical diagram is illustrated in FIGURE 8. Energy from the source is focused by a pivoted mirror onto a diagonal plane mirror, through the entrance slit and into the monochromator. Dispersed radiation from the prism is focused at the exit slit and passes into the sample compartment. Here the beam is alternated between sample and reference beams at 35 cps by a vibrating mirror assembly, and the alternating pulses of energy are focused on a 1P28 photomultiplier. The sample beam signal is continuously compared with a fixed reference beam signal and the ratio is read out as transmittance on a meter. Readout may also be transferred to a potentiometric recorder, if desired, in which case the instrument becomes a true ratio recorder.

The sources used are both quite conventional. A hydrogen discharge lamp is used for the region 220 to 320 mμ, and a tungsten lamp for 320 700 mμ.

The monochromator is basically the same as the DU monochromator, with the following exceptions:

(1) It is only three fifths as large.

(2) The prism is fused silica instead of natural crystalline quartz. In this wave length range, fused silica was chosen over natural quartz because of transmittance characteristics and cost, and over synthetic quartz because of cost and availability.

(3) The slits are cam-programmed with a cam override to allow manual adjustment.

The monochromator slit adjustment is by a mechanically operated cam program which varies the slit as a function of wave length along two predetermined programs; the narrow program, which is the most commonly used, and the medium program, which provides a factor of two increase in energy for differential analysis in strongly absorbing solvents. In addition, a manual program is provided for flame photometry work. In this mode of operation, a micrometer adjustment provides continuous slit adjustment from 0 to 2 mm.

The normal operating mode is double beam, although a single beam mode is also provided for flame photometry. The double beam mode was selected to provide continuous compensation for reference solvent absorption and 100 per cent T line adjustment, and to allow the operator to make rapid survey measurements by simply turning the wave length dial and observing the meter. Since these two operations normally consume about 60 per cent of total measurement time, a considerable saving is effected.

Beam alternation is done by a vibrating mirror assembly that oscillates at a self-resonant frequency of approximately 35 cps. A detailed view is in FIGURE 9. This assembly consists of 4 gold-plated steel reeds

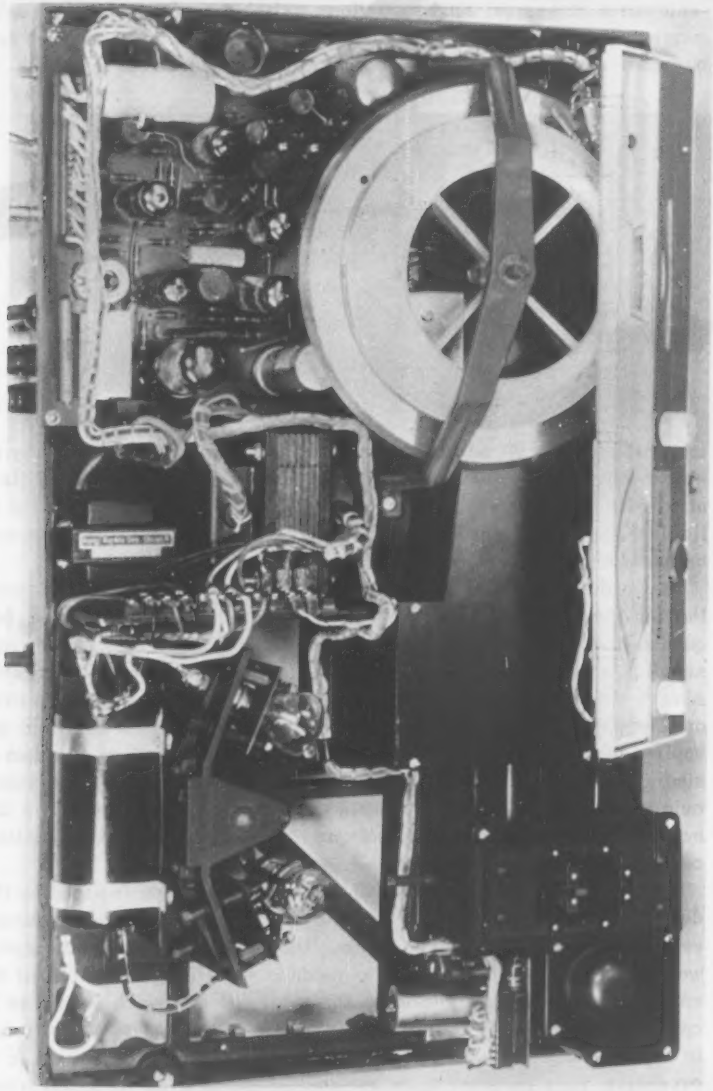

FIGURE 7. Model DB component layout

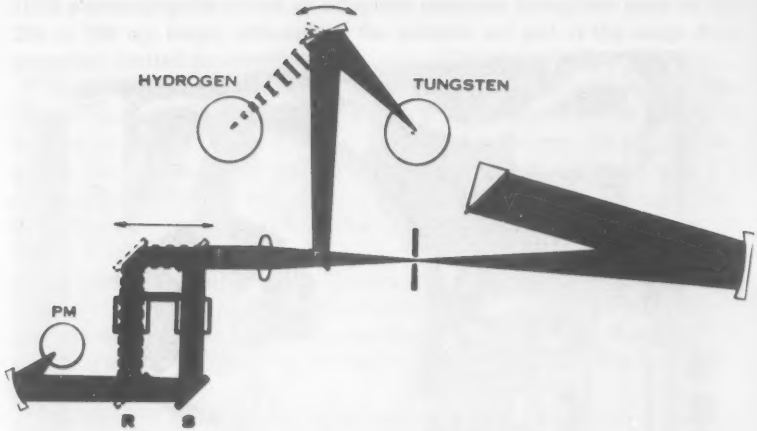

FIGURE 8. DB optical diagram

fastened to a mirror platform that is driven at its natural frequency by an electromagnet in the base. Allowing the vibrating assembly to oscillate at its natural frequency makes its operation completely independent of line frequency and insensitive to small variations in mass from one bridge to another.

While the masses of all vibrating mirror assemblies are not exactly the same, they all vibrate at a frequency close to 35 cps. The exact frequency is actually immaterial, since the bridge generates its own driving signal by means of a small photocell mounted in the base of the assembly. A vane attached to the vibrating assembly modulates a light beam falling on the photocell, producing an in-phase signal that is amplified and applied to the drive coil. Another photocell-vane mechanism operates in similar fashion to produce an out-of-phase signal that limits the amplitude of the oscillation. The sample compartment is a casting that fits between the diagonal vibrating mirrors and which will admit absorption cells up to 40 mm. in length.

Limitation of sample path to 40 mm. is a restriction imposed by the design of the vibrating mirror assembly. In order to incorporate sample cells appreciably larger than 40 mm., the mass of the bridge assembly would have to be so great as to produce significant vibration of the entire instrument. In addition to this, a frequency much less than 35 cps which would be a consequence of the increased mass would result in a pulsation effect on the transmittance meter and would result in an excessive time constant when the instrument is used to follow a fast reaction or transmittance change.

Oscillation of the mirror assembly produces alternating pulses of light from sample and reference beam as shown in FIGURE 10. The

alternating pulses are switched either to the reference regulating amplifier or to the metering circuit by a relay in phase with the vibrating mirror assembly. The regulating amplifier circuit represents a combination of optical and electrical compensation. The mechanical slit program is designed to compensate for source energy and detector response

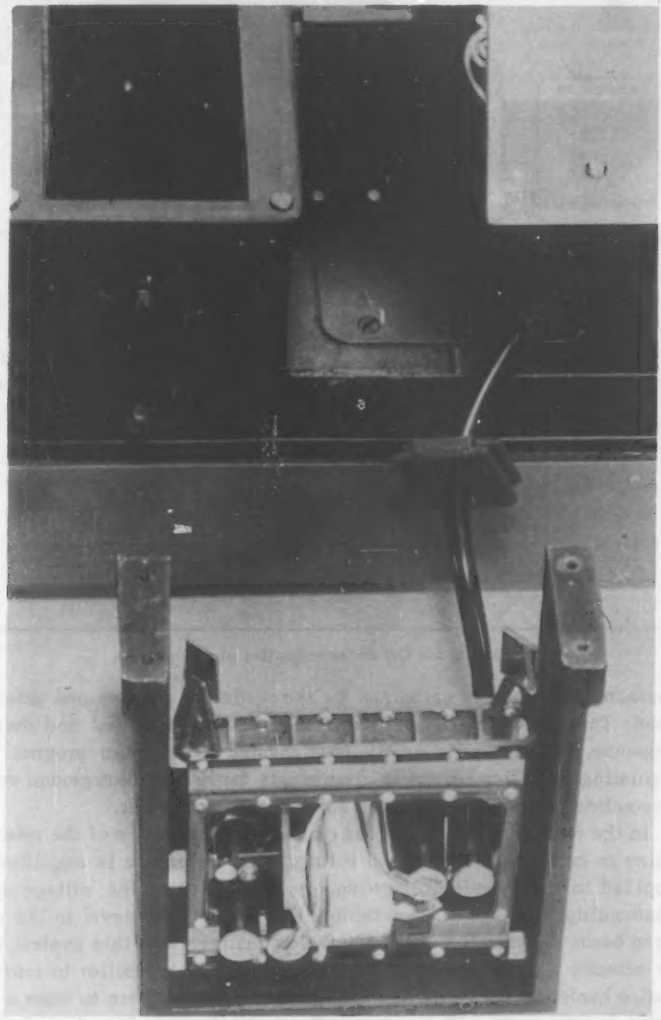

FIGURE 9. Vibrating mirror assembly

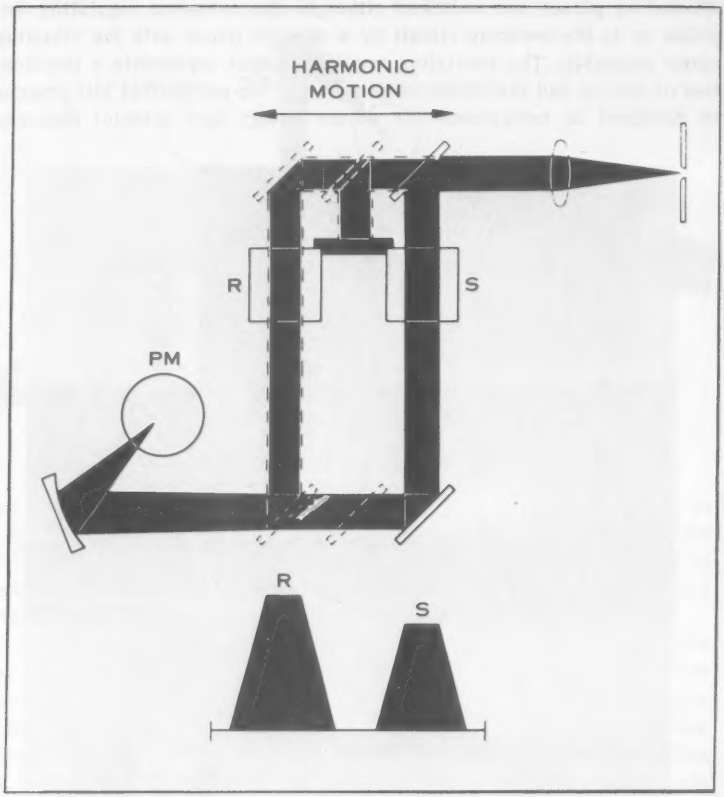

FIGURE 10. DB photomultiplier signal pattern

characteristics and is optimized for the particular sources and detectors used. Thus predictable variations, such as source output and detector response, may be quite nearly compensated by the cam program. The regulating amplifier serves to compensate further for background energy fluctuations caused by reference solvent absorption, etc.

In the regulating amplifier (FIGURE 11) the amplitude of the reference pulse is compared with a fixed voltage. The difference is amplified and applied to the dynode voltage supply, which varies the voltage on the photomultiplier dynodes, maintaining the 100 per cent level in the reference beam. A dynamic gain of 100,000 is realized with this system, which is actually enough to enable the dynode voltage controller to carry the entire burden of compensation. For instance, if one were to scan a wide wave length range with a narrow, fixed slit (as is often done in flame photometry) the slit program would be completely inoperative and all

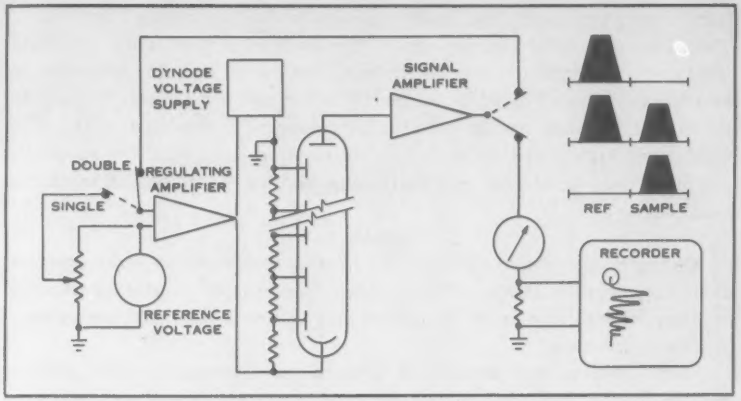

FIGURE 11. DB electronic diagram

compensation would have to be done electrically. In practice, however, this is not usually done, since dynode voltages approaching 1000 could be applied to the photomultiplier, causing it to become quite noisy.

Application of the dynode voltage controller to a rather extreme case of energy variation is illustrated in FIGURE 12. The lower curve is

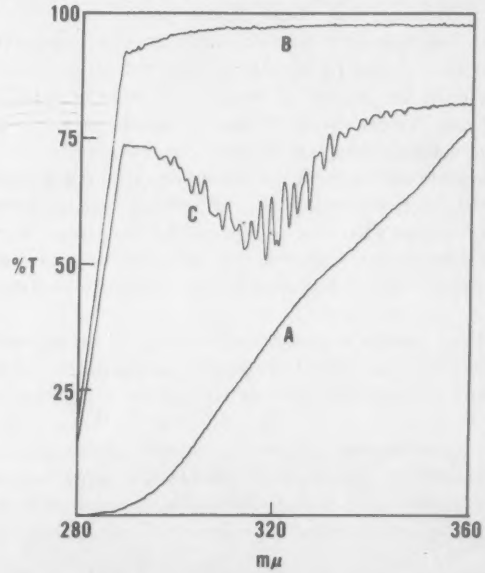

FIGURE 12. Differential analysis curve. (A) Butyl ether versus iso-octane; (B) butyl ether versus butyl ether; (C) as above plus. CS_2 vapor on sample side.

butyl ether versus air. The upper curve is butyl ether versus butyl ether. The excursion of the 100 per cent T line is the point at which no further electrical compensation may be accomplished. Note that this corresponds to a transmission of about 2 per cent T in the reference beam. The middle curve is CS_2 vapor on the sample side along with the butyl ether. The significant aspect of this is that all three were made with the same slit settings, thus providing reproducible resolution regardless of reference beam energy.

Summary

We have discussed some of the factors that must be considered in designing and building a UV-visible near-infrared spectrophotometer and how these have been integrated into a newly designed instrument. To summarize them:

First, source and detector at present pose no particularly serious problems. It is, however, necessary to use two sources and two detectors in order to get satisfactory performance throughout the UV-visible near-infrared region.

At present, combinations of the tungsten or hydrogen lamps as sources, and the photomultiplier or PbS cell as detectors, represent by far the most common components used in commercial instruments.

Selection of a dispersing element carries with it the necessity for fundamental decisions on other design features. Use of a pure grating monochromator dictates that steps should be taken to eliminate stray radiation from overlapping orders. In addition, the linear dispersion or constant band pass commonly employed does not allow optical compensation for fluctuations in source output and detector sensitivity. Consequently, it may be necessary to use automatic gain control or speed suppression to maintain adequate system response.

Prism monochromators must be equipped with some system of slit programming to compensate for not only source and detector variation, but also for nonlinear dispersion produced by the prism. In this system, the optical compensation from the slit variation may be aided by automatic gain control, speed suppression, or automatic control of detector sensitivity.

Design of the sample compartment is to some extent dictated by the optical system employed, and it influences considerably the size, nature, and the desired applicability or versatility of the instrument with respect to ancillary devices.

The readout mechanism is chosen primarily on the basis of accuracy and speed desired and significantly affects the range versatility of the instrument. Various systems of recording, scale expansion, and data presentation may be incorporated to expand the usefulness of the entire instrument.

Conclusion

I have hoped, by these few remarks, to convey some of the essentials

of instrument design philosophy and the limitations that dictate what instrument manufactuerrs must consider in designing an instrument for wide useage. Perhaps these comments may be of value in understanding the factors that should be considered in evaluating and selecting instruments for use in the ultraviolet, visible, and near-infrared spectral regions.

Acknowledgments

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DIVISION OF MICROBIOLOGY

ANTIBIOTICS IN EXPERIMENTAL CANCER CHEMOTHERAPY*

Morris N. Teller

Division of Tumor Biology, Sloan-Kettering Institute for Cancer Research New York, N. Y., and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York, N. Y.

Antibiotics in Experimental Cancer Chemotherapy

The discovery of antibiotics initiated a new era in the history of chemotherapy, one in which the arsenal of the physician was greatly increased by substances effective against bacterial, fungal, and protozoan diseases, the treatment of which was heretofore limited. Successful therapy of such diseases naturally aroused intense interest in the possible utility of such materials in the treatment of cancer. The specific nature of antibiotics, the ability to inhibit one group of microorganisms without affecting a closely related group, indicated the possibility of differentiating similarly between normal and neoplastic cells.

Early investigations in which various antibiotic preparations were used for cancer chemotherapy have been reviewed by Reilly.[1] Many believed that the results seen were not necessarily due to direct effects on tumor cells, but that improvements in the general condition of the host were due to the control of bacterial infections by the antibiotics. More recent investigations have shown that direct antitumor action is possible. These suggestive temporary antitumor effects obtained in laboratory animals and in cancer patients by the use of such compounds spurred the search for other and more effective anticancer agents derived from microbiological products. In recent years, a program was developed by the Cancer Chemotherapy National Service Center, Public Health Service, Bethesda, Md., to implement research in this area.[2] At the present time, many thousands of fermentation products are being screened for anticancer activity in various laboratories under its sponsorship.

A large number of systems have been devised for the testing of materials for antineoplastic activity, including various tumor and nontumor systems.[3] Among these, and of more recent origin, are those using transplantable human tumors growing in laboratory animals. The testing of various antibiotics for antineoplastic activity in the human tumor-rat host experimental chemotherapy systems will be discussed here.

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Although human neoplastic tissue has been transplanted successfully to various animal sites such as the eye and the brain of normal (nonconditioned) heterologous hosts, there were many deterrents to their wide application and use as bioassay systems for testing potential anticancer agents. Toolan, by "conditioning" the host with X radiation and/or cortisone, demonstrated the feasibility of transplanting human tumors for many generations and maintaining them in large numbers of rats and hamsters.[4] This method was modified and standardized for experimental chemotherapy usage.[5] Details of the method for using transplantable human tumors in experimental chemotherapy have been published elsewhere.[5,6] For convenience, a brief description follows.

Random-bred weanling female Wistar and Sprague-Dawley albino rats were X-irradiated with a single total-body dose of 150 r 1 to 3 days prior to transplantation of tumor tissue. The transplantable human tumors used were the sarcoma H.S. No. 1 and the epidermoid carcinoma H.Ep. No. 3, descriptions of which have been published.[4] For chemotherapy trials, 0.5 ml. of a 50 per cent suspension of tumor tissue was injected subcutaneously in the right flank of the X-irradiated rat. Rats implanted with aliquots of the same tumor tissue were divided into groups of 5 to 12 per treatment, one group being used as controls. Immediately after transplantation of the tissue, the rats were given a subcutaneous injection of cortisone acetate near the nape of the neck, at a dose of 60 mg./kg. of body weight. Rats bearing H.S. No. 1 tumor were given injections again on alternate days for a total of 4 doses; those bearing H.Ep. No. 3 tumors were given cortisone injections at 3- to 4-day intervals for a total of 3 doses. Therapy was started 24 hours after tumor transplantation and continued as single, daily intraperitoneal (IP) injections (Sundays excepted) for a total of 9 injections for H.S. No. 1 and 7 to 8 injections for H.Ep. No. 3. Oral treatment (PO) was by intubation once daily.

Experimental H.S. No. 1-bearing rats were sacrificed on the 11th or 12th day, and those bearing H.Ep. No. 3 were sacrificed on the 9th or 11th day, 24 to 48 hours after the last dose of drug had been given. The tumors were excised, blotted, and weighed after removal of necrotic material. Two or more tests at the same dose were pooled to give an average effect; where only two results were available for a particular dose, and results did not agree, these were listed separately. A test was designated "lethal" if host mortality was $\leq$ 50 per cent, and "toxic" if the average weight change of the treated rats was $\leq$ -6gm. and/or host mortality reached 2/6, or if the rats were in a debilitated condition; doses not producing lethality or toxicity were considered "tolerated."

For H.S. No. 1, tumor inhibitions of 0 to 39 per cent were graded - (negative); 40 to 74 per cent, $\pm$; 75 to 99 per cent, +; and 100 per cent, ++. With respect to H.Ep. No. 3, tumor inhibitions of 0 to 24 per cent were graded -; 26 to 59 per cent, $\pm$; 60 to 99 per cent, +; and 100 per

cent, ++. The graded effects were based upon the extent of variability discussed previously.[5]

The compounds were prepared as solutions in physiological saline or as suspensions in 0.5 per cent carboxymethylcellulose such that the dose was administered in 0.5 to 0.6 ml. Stock solutions of the actinomycins were prepared in absolute ethyl alcohol in concentrations of 0.5 mg./ml., and diluted as necessary with physiological saline. Except for P5995-9, the original letter designations for the actinomycins were retained. P5995-9 is the Sloan-Kettering designation for a crystalline preparation tentatively identified chromatographically as resembling actinomycin A*.

Our interest in microbiological products led to the testing of a number of crude culture filtrates for antitumor activity. These were received in a frozen state and kept so until immediately before use. The filtrates were thawed in a water bath at 37° C. and either used full strength or diluted accordingly with sterile distilled water if toxicities were known. Three ml. of solution were injected IP twice daily in equally divided doses six hours apart.

The number of active preparations found in tests of crude culture filtrates was small. For example, in a series of 156 broths submitted, 180 tests were required. Of these, repeated activity was obtained in 2 samples and confirmed in only 1 of the refermentations. Loss of activity on storage of filtrates, even at -70° C., or on refermentation of the original culture, is commonly encountered. For these and other reasons, few materials reach the final stage of tests as pure crystalline products. Many are discarded at some stage of purification because of extreme toxicity, or upon recognition as a known compound, or for various other reasons.

Following our interest in compounds of microbiological origin, a number of crystalline antibiotics were tested for antitumor activity in the human tumor-rat host experimental chemotherapy systems. Results of tests with nine antibiotics are given in TABLES 1 (H.S. No. 1) and 2 (H.Ep. No. 3). The compounds were injected intraperitoneally.

Streptonigrin inhibited the growth of H.S. No. 1 to a + degree; a similar + inhibition by actinobolin was accompanied by host toxicity. (TABLE 1). Inhibitory effects seen with toxic doses are considered of questionable validity, for a reduction in tumor growth can (but not necessarily) result from host toxicity, rather than from the direct action of test compound on tumor cells. Although a + effect was obtained with fumagillin at 6.25 mg./kg., the toxicity encountered in the test at 3.13 mg./kg. indicated that the higher dose might be toxic in additional tests. Plus-minus (±) effects against H.S. No. 1 occurred with actidione and streptimidone. As with fumagillin, mitomycin C was toxic at 0.38 mg./kg.

*Personal communication, Ralph K. Barclay, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

TABLE 1
EFFECTS OF VARIOUS ANTIBIOTICS ON H.S. NO. 1 GROWING IN THE CONDITIONED RAT

Compound	Dose (mg./kg./day)	Toxicity: Av. host wt. Δ (gm.) Treated	Control	Mortality Treated	Control	Av. tumor wt. (gm.) Treated	Control	Effect
Actidione	0.8	+18	+20	2/6	0/6	3.0	6.4	± toxic
	0.4	+10	+14	0/6	0/6	3.4	5.8	±
	0.2	+ 5	+10	0/8	3/8	7.4	7.6	-
Azaserine	2.6	- 3	+14	2/12	0/12			toxic*
	2.0	+ 8	+19	0/12	1/12	4.6	6.9	- * †
	1.3	+16	+17	0/6	0/6	5.4	7.1	-
Mitomycin C	0.75	+ 2	+12	1/12	0/12	1.5	4.2	± *
	0.38	-10	+ 6	0/6	2/6	2.7	6.5	± toxic
	0.19	+ 2	+12	0/6	0/6	4.1	5.6	-
Actinobolin	1000.	+ 5	+22	2/6	0/6	1.1	4.4	+ toxic
	500.	+ 1	+ 1	0/6	0/6	1.7	4.8	±
	250.	+23	+22	0/6	0/6	2.5	4.3	±
	125.	+15	+11	0/6	1/6	3.2	1.7	-
Streptonigrin	0.5	-13	+ 6	2/6	1/6			toxic
	0.25	- 4	+11	0/6	0/6	0.3	4.2	+
	0.125	+ 3	+11	0/6	0/6	1.3	4 2	±
	0.065	- 2	+12	1/12	0/12	0.7	6.8	+ *
	0.033	+ 7	+ 9	0/6	0/6	1.9	8.6	+
Sarkomycin	1000.			6/6	0/6			Lethal
	500.	+ 2	+13	0/6	0/6	4.6	6.6	-
6-Diazo-5-oxo-norleucine	0.05	- 7	+ 5	1/8	2/8	4.0	4.1	-
Fumagillin	6.25	- 1	+12	1/8	0/8	1.0	7.6	+
	3.13	- 7	- 1	0/5	0/5	1.9	5.0	± toxic
	1.6	+ 2	0	3/17	0/17	1.9	6.2	± *
	0.8	+14	+18	0/6	0/6	6.0	4.6	-
Streptimidone	50.	+ 6	+18	0/6	0/6	2.8	6.7	±
	25.	+ 8	+15	0/12	0/11	2.8	6.9	± * †
	12.5	+ 1	+ 3	2/10	1/10	2.6	6.0	± *

*Average of pooled results for two or more tests.
†Toxic in one test.

(± effect), but not in the single test at 0.75 mg./kg. (± effect). Azaserine, sarkomycin, and 6-diazo-5-oxonorleucine were inactive against H.S. No. 1.

Tests against H.Ep. No. 3 yielded + effects with actinobolin, streptonigrin, and fumagillin, while ± effects were obtained with actidione, azaserine, mitomycin C, and streptimidone (TABLE 2). Sarkomycin and 6-diazo-5-oxonorleucine were inactive against H.Ep. No. 3, as they were against H.S. No. 1.

It is not surprising that seven of the nine antibiotics tested were active against one or the other of the human tumors, for their selection was based on their activities against various animal tumors. This is not to be taken as an absolute indication of similarities between the animal tumor and the human tumor systems. Such a comparison is presented elsewhere.[6] The data given here affords additional evidence of the potential tumor-inhibiting properties of antibiotic preparations.

Other antibiotics, such as the actinomycins, have also been of interest as a class of compounds with known antitumor agents. Since the discovery and isolation of actinomycins from *Streptomyces antibioticus*

TABLE 2

EFFECTS OF VARIOUS ANTIBIOTICS ON H.EP. NO. 3 GROWING IN THE CONDITIONED RAT

Compound	Dose (mg./kg./day)	Toxicity: Av. host wt. Δ (gm.) Treated	Control	Mortality Treated	Control	Av. Tumor wt. (gm.) Treated	Control	Effect
Actidione	0.8	+ 6	+13	1/6	0/6	1.4	1.9	±
	0.4	+ 5	+ 8	1/8	1/8	2.3	4.1	±
	0.2	+ 5	+ 8	0/8	1/8	4.0	4.1	-
Azaserine	2.6	- 3	+ 5	2/30	0/30	2.0	3.4	± * †
	2.0	+10	+12	0/6	0/6	3.4	1.6	-
Mitomycin C	0.75	- 3	+16	0/12	1/12	2.3	3.9	± * †
	0.38	+11	+18	0/6	1/6	4.5	5.2	-
Actinobolin	1000.	+ 3	+ 9	0/8	1/8	1.1	4.6	+
	500.	+ 4	+ 5	0/6	1/6	1.8	4.2	±
	250.	+21	+15	1/6	0/6	5.8	3.8	-
Streptonigrin	0.25	- 4	+13	0/16	0/16	0.2	1.9	+ * †
	0.13	- 2	+ 8	1/16	1/16	1.2	2.8	± * †
	0.065	+ 3	+12	0/18	0/18	1.1	3.6	+ *
Sarkomycin	1000.	0	+12	3/6	0/6			lethal
	500.	+15	+11	0/5	0/5	2.6	3.4	-
6-Diazo-5-oxo-norleucine	0.05	0	+ 6	0/6	0/6	3.9	4.4	-
Fumagillin	6.25	- 4	+ 7	0/16	0/16	0.6	2.2	+ *
	3.13	- 4	- 6	1/13	3/14	2.3	2.3	- *
Streptimidone	25.	- 2	0	3/12	1/12	2.3	4.3	± *
	12.5	+ 4	+ 2	0/16	1/16	1.5	3.2	± *

*Average of pooled results for two or more tests.
†Toxic in one test.

by Waksman and Woodruff,[7] and the reported selective cytostatic activities of actinomycin A on mammalian tissue,[8] wide interest has been shown in these compounds as possible antineoplastic agents. Results of preliminary tests with several actinomycins in the human tumor-rat host systems led to the testing of a larger series of these compounds in the search for antitumor activity. Results of tests with four actinomycins, C, D, J, and P5995-9 are presented in TABLES 3 and 4; the route of administration was IP.

Only actinomycin P5995-9 was active (± effect) at a tolerated dose in tests against H.S. No. 1 (TABLE 3). Plus-minus (±) effects were obtained with actinomycins C and D, but at doses in which at least one test was toxic. Actinomycin J was inactive against H.S. No. 1.

Actinomycins C and D gave ± effects against H.Ep. No. 3, and both + and ± effects were obtained with actinomycin J (TABLE 4). At 0.1 mg./kg., one of three tests with P5995-9 was toxic, the other two tests averaging a + effect; the inhibitory effect was ± at 0.05 mg./kg.

Since the route of administration of a drug may affect its activity, the four actinomycins were administered orally to rats implanted with H.Ep. No. 3. Results are given in TABLE 5.

It is readily apparent that the results obtained for orally administered actinomycins are different from those obtained in tests where the compounds were injected intraperitoneally. Larger amounts of compound were

TABLE 3

EFFECTS OF FOUR ACTINOMYCINS ON H.S. NO. 1 GROWING IN THE CONDITIONED RAT

Actinomycin	Dose (mg./kg./day)	Toxicity: Av. host wt. Δ (gm.) Treated	Control	Mortality Treated	Control	Av. tumor wt. (gm.) Treated	Control	Effect
C	0.06	-16	+ 8	0/6	0/6			toxic
		+ 3	+10	0/12	0/12	2.6	4.6	± *
	0.03	+ 6	+10	0/5	0/5	7.3	7.8	-
D	0.03	-11	- 2	2/5	0/5			toxic
		+ 3	+ 9	10/41	5/42	3.0	6.6	± *
	0.015	+ 3	+ 5	0/6	0/12	6.4	7.8	-
J	0.06	- 6	+10	7/12	0/12			lethal
	0.03	- 1	+ 3	2/15	0/15	3.4	3.9	- *
P 5995-9	0.1	- 6	+18	1/12	1/12			toxic *
	0.05	+ 6	+17	0/12	0/12	1.6	5.3	±
	0.025	+10	+11	0/6	1/6	4.6	1.7	-

*Average of pooled results for two or more tests.

tolerated and, except for actinomycin D, the antitumor effects against H.Ep. No. 3 were obtained over a wider range of tolerated doses. No tumors grew in rats treated orally with actinomycin C at 4 mg./kg., and + effects occurred with actinomycins J and P5995-9.

A comparison was made among the tumor-inhibiting actinomycins based on the ratio Maximum Effective Dose-minimum effective dose (MED/med). The MED as used here is defined as the maximum "tolerated" (nontoxic) dose-producing tumor inhibition, and med as the minimum nontoxic dose yielding a ± effect. Since a doubling of the MED generally caused toxicity and/or lethality, the MED/med ratio approximately represents a therapeutic index.

TABLE 4

EFFECTS OF FOUR ACTINOMYCINS ON H.EP. NO. 3 GROWING IN THE CONDITIONED RAT

Actinomycin	Dose (mg./kg./day)	Toxicity: Av. host wt. Δ (gm.) Treated	Control	Mortality Treated	Control	Av. tumor wt. (gm.) Treated	Control	Effect
C	0.12	- 9	+22	0/6	0/6			toxic
	0.06	+ 4	+ 6	0/21	0/22	1.6	3.3	± *
	0.03	+ 1	- 1	2/5	1/5	3.7	3.4	-
D	0.06	- 2	+ 5	5/24	4/24	2.3	5.3	± *
	0.03	+ 1	+ 4	3/58	5/57	2.6	4.6	± *
	0.015	+ 8	+ 7	1/8	0/8	6.8	6.3	-
J	0.12	-10	+11	2/12	0/12			toxic *
	0.06	- 5	+ 9	0/5	0/5	2.4	4.6	±
	0.03	+ 1	+ 2	0/10	1/10	1.7	4.2	+ *
	0.015	+10	+19	0/6	1/6	1.9	4.8	+
	0.008	+10	+19	0/6	0/6	1.9	4.0	±
	0.004	+15	+10	0/6	0/6	2.1	1.5	-
P5995-9	0.1	- 6	+13	0/6	0/6			toxic
		+ 1	+22	1/12	1/12	0.3	4.8	+ *
	0.05	+10	+15	0/24	0/24	2.3	4.7	± *
	0.025	+15	+22	0/6	0/6	4.1	4.1	-

*Average of pooled results for two or more tests.

TABLE 5

EFFECTS OF ORALLY ADMINISTERED ACTINOMYCINS ON H.EP. NO. 3 IN THE RAT

Actinomycin	Dose (mg./kg./day)	Toxicity: Av. host wt. Δ (gm.) Treated	Control	Mortality Treated	Control	Av. tumor wt. (gm.) Treated	Control	Effect
C	4.0	- 2	+ 5	0/6	0/6	0	2.5	++
	2.0	+ 3	+ 5	0/6	0/6	0.5	2.5	+
	1.0	+ 4	+ 5	0/6	0/6	1.4	2.5	±
	0.5	+ 8	+ 5	0/6	0/6	1.4	2.5	±
	0.25	+18	+26	0/6	0/6	5.3	6.0	-
D	2.0	- 9	+10	1/6	0/6			toxic
	1.0	+ 5	+17	0/6	0/6	1.0	1.8	±
	0.5	+ 9	+15	0/12	0/12	1.5	3.6	± *
	0.25	+ 5	+10	0/6	0/6	1.5	1.3	-
J	2.0	+ 2	+17	0/6	0/6	0.4	1.8	+
	1.0	+13	+22	1/12	0/12	2.5	4.9	± *
	0.5	+17	+22	2/12	0/12	2.3	4.9	± *
	0.25	+18	+15	0/6	0/6	4.3	6.9	±
	0.13	+15	+15	1/6	0/6	3.2	6.9	±
P5995-9	4.0			6/6	0/6			lethal
	2.0	+ 9	+13	0/12	0/12	2.1	5.8	+
	1.0	+10	+21	0/6	0/6	1.1	5.7	+
	0.4	+10	+18	1/12	0/11	1.7	4.8	+ *
	0.1	+23	+18	0/12	0/12	2.0	5.1	+ *
	0.05	+14	+17	0/12	0/12	4.2	3.7	- *

*Average of pooled results for two or more tests

MED/med ratios for the actinomycins tested against H.S. No. 1 (TABLE 3) could not be calculated, for activity, when present, was at one level only. On the other hand, the antitumor activity of some actinomycins against H.Ep. No. 3 permitted calculations for MED/med ratios. A comparison between such indexes for intraperitoneally and orally administered actinomycins tested against H.Ep. No. 3 is given in TABLE 6. It is apparent that, except for actinomycin D, oral treatment with actinomycins C, J, and P5995-9 offers an advantage; a twofold or greater difference in MED/med indexes is thus obtained. Intraperitoneally injected actinomycins C, J, and P5995-9 gave MED/med indexes of <1, 7.5, and <1, compared to indexes of 8, >15, and 20, respectively, for these compounds administered orally.

The usefulness of the actinomycins as antitumor agents is tempered to a large degree by their general body toxicities. On the other hand, the high MED/med indexes for orally administered actinomycins C, J, and

TABLE 6

COMPARISON OF MED/MED INDEXES FOR INTRAPERITONEALLY AND ORALLY ADMINISTERED ACTINOMYCINS IN TESTS AGAINST H.EP. NO. 3

Actinomycin	MED/med IP	PO
C	<1	8
D	2	2
J	7.5	>15
P5995-9	<1	20

P5995-9 indicate that toxic properties of this class of compounds may differ considerably. It is possible that the further elucidation of the structural configurations of the various actinomycins,[9] and the biosynthesis of new actinomycins,[10] may make available compounds with less toxicity and greater tumor-inhibiting properties.

It is evident that products of microbial fermentations provide an excellent source of potentially useful antitumor agents. However, many of these are highly toxic, as disclosed occasionally in auxilliary tests[11] and provide negligible differential toxicities between normal and neoplastic cells. The ability of microorganisms, however, to synthesize an apparently unlimited number of different compounds provides the hope that one or more useful antineoplastic agents may be disclosed in the future.

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DIVISION OF OCEANOGRAPHY AND METEOROLOGY

THE CONTROLLED-CLIMATE CHAMBER FOR STUDY OF THE EFFECTS OF METEOROLOGICAL CHANGES ON HUMAN DISEASES*

Joseph Lee Hollander
University Hospital, University of Pennsylvania, Philadelphia, Pa.

Empiricism has characterized the tremendous number of studies on the effects of weather upon human disease. Arthritic patients in particular have claimed to feel impending weather changes by a worsening of joint pain and stiffness. This has been so well known that such persons have been widely regarded as weather prophets, some to a degree of accuracy that their predictions are almost as dependable as the United States Weather Bureau. Since neither medicine nor meteorology are as yet exact sciences, however, there is still room for improvement.

Animals can foretell a storm by some unknown sense, long before there are obvious changes in wind direction, barometric fall, or other known factors. Seagulls flock to inland waters before any severe storm occurs. Even insects seem able to adjust their habits to climatic variables. Any of us who has had a severe bone fracture, a large operative scar, an amputation, or any severe injury may well feel uncomfortable sensation in the site of the old injury when frontal conditions approach. No one has yet discovered how or why this is possible, nor is it known what factor or factors of weather change is responsible.

Observers have agreed for many years that there is a definite "weather effect" on rheumatism or arthritis. Even from personal experience during the past 4 years since my own first attack of arthritis, I must agree that this "weather effect" is not just another old wives' tale. Since all such observations, including my own, are strictly empirical, it is high time that controlled scientific observations be made to determine whether this effect is reproducible, what factors are necessary to induce "weather effect," and finally what happens in the organism when such conditions occur.

A review of the scientific literature on the effect of climate on human diseases is discouraging indeed. Whole library shelves are filled with volumes on the subject, but little except conjecture superimposed upon empirical observation is to be found. In reviewing the literature on the effect of barometric pressure variations on humans, Dordick[1] wisely concludes that there is little to prove that even marked barometric changes have any unique effect on the human body, and adds that the well-known

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changes in symptoms experienced by many on the approach of weather changes cannot be attributed to barometric change (or any other single factor) since "total weather change" includes at least several psychrometric variations simultaneously.

Chilling has been condemned for centuries as the factor primarily responsible for worsening of arthritic symptoms[2]. This is not a single climatic factor, but rather the resultant effect upon a patient of decreased temperature, and/or decreased or increased humidity, and/or increased rate of air movement. Even with very high air temperature and humidity chilling is frequent from turning on an electric fan (*i. e.* increasing rate of air movement) upon exposed areas of the body.

Much of the recent literature on this subject has been concerned with the degree of ionization of the air.[3] Methods of producing and counting charged particles in the air have improved tremendously during the past few years, and much interest has been aroused by reports that negative ionization exerts an ameliorating effect on symptoms, whereas positive ionization often aggravates the complaint and has other deleterious effects. The marked shifts in air ionization occuring before and during a thunderstorm are widely known, and have added to the zeal of those who would explain the "weather effect" simply on the basis of ion changes. The experimental methods of nearly all of the investigators in this field leave some doubt in the minds of the critical concerning the validity of conclusions reached, since the experiments used subjects in full view of the ionizing and counting apparatus, and used controls without turning on the the equipment. This approach completely ignores the human suggestibility by which many of us feel better with the administration of completely inert chemical substances in a so-called "placebo" tablet. Short-term observations are notoriously inaccurate for this reason, and even repeated daily sessions may yield inaccurate conclusions. Only when patients are continuously observed over a long period without any visible or audible evidence of apparatus being turned on, in other words, only when the experiment is a long-term "blind" study, can the results be accurately determined.

Cited above are the reasons why I felt it necessary to have facilities for complete control of all possible climatic factors for study of "weather effect" on human disease. Variations must be completely "blind" to the subjects, who are confined for long periods continuously in order to allow for adjustment and time for conducting a series of objective tests during and after various climatic variations. Such an apparatus would make possible the control of temperature, humidity, barometric pressure, rate of air movement, and air ionization, but could not solve completely the problem of objectivity of results on the patients. It could only minimize the chances of error due to human reactions.

The idea of a controlled-climate chamber is not new. There have been such facilities for study of plant and animal life for some years, and laboratories for study of extremes of environmental conditions on humans are

well known, Edstrom[4] probably was the first to construct a controlled-climate chamber for long-term occupancy by humans with arthritis. His chamber was used only for study of the effects of a continuous warm, dry climate on these subjects, and no facilities were available to vary the conditions or to control barometric pressure, ionization, or rate of air movement in the chamber. His results did show that the arthritic seemed better off in various ways as a result of confinement in the chamber for periods up to three months with temperature at 84° F. and humidity at 40 per cent. Since these results were published nearly 15 years ago practically nothing has been added.

The task of constructing our controlled-climate chamber was extremely difficult. We had to raise $125.000 to build it, even after finding an engineer with sufficient ingenuity to believe it was possible to translate this dream into reality. This ingenious engineer will describe in a subsequent presentation some of the technical difficulties that he surmounted in this complicated and unique construction.

The controlled climate chamber, or "climatron," is situated in the Hospital of the University of Pennsylvania. It has been in almost continuous use since it was completed in January 1961. The facility consists of a 15-foot square room equipped for continuous living and sleeping by two patients at a time, with a connecting bathroom, and an air-lock vestibule for entrance and exit. Walls, floor, and ceiling are constructed of reinforced concrete 10 in. thick to withstand the pressure variations. The window is completely sealed, and consists of two layers of heavy plate glass. The room is attractively decorated and furnished, as a hotel studio-twin room might be, but with a wall decorated with a mural, and a large mirror to avoid any feeling of claustrophobia. The conditioned air is pumped into the chamber through multiple tiny perforations in the false ceiling, and exhausted through a duct near the floor. The bathroom appears fairly standard, but special facilities were required for drainage because of pressure differences between the chamber and the outside atmosphere. These will be described later.

The airlock vestibule also called for considerable ingenuity for construction. The submarine doors that seal the chamber to the vestibule, and the vestibule to the outside corridor are perforated by small windows and air valves for equalizing pressure before each is opened. One door must be sealed before the other is opened.

Automatic recording instruments on the control panel outside the chamber write a continuous record of temperature, dew point temperature, barometric pressure, rate of air movement, amount of fresh air taken into the system, and the charge and concentration of ions in the room air of the climatron. Controls for programming the desired changes in temperature, humidity, and barometric pressure in advance are also placed in the control panels, as well as alarm mechanisms that signal any departure from the prescribed pattern. Ionization of the room air is generated by

coronal discharge of high voltage from three small needle-ionizing heads in the ceiling of the chamber powered by the generator in the control panel. Counting of the ions is made by an Ebert ion counter mounted in a small cabinet in the center of the room, and the count is recorded continuously on a kymographic chart on the control panel.

It would have been easier and cheaper to place much of the apparatus in the room, but I insisted throughout that the patients must live in a *room,* and not a laboratory, since we must eliminate the psychologic effect of seeing the apparatus about the room. The ion counter cabinet, and the small ionizing heads in the ceiling are the only visible apparatus in the room. Except for these and the submarine door to the airlock the chamber appears like any hotel room of modern vintage.

All the complicated machinery for processing the air for the Climatron is housed in a room below the chamber. The description of this room I shall leave to the engineer who designed it and understands it far better than I.

Having described the apparatus in a truly amateurish manner, I can now proceed to matters that I understand better. The Climatron permits me to keep all the variables constant in the chamber, or to vary them singly or in combination at predetermined rates and amounts, completely without the knowledge of the patients housed in the room. With the programming devices, changes can take place even during the night, and certainly independent of any visitations by me, my assistants, nurses, or any other attendants.

Two patients at a time live continuously in the chamber for periods of at least 3 weeks. Meals and supplies are brought in through the airlock. The patients have a telephone to the nurse's station and to the outside world. They are permitted to have visitors as any hospital patients. One of our patients even acted as a babysitter each evening for her granddaughter while her daughter and son-in-law attended evening classes a block away in the university. The visitors soon learn to operate the airlock doors themselves, but the patients do not use them.

The patients are entertained by their radio (AM and FM), television, and by arts and crafts sypervised by our occupational therapy department. Since the continuous incarceration makes adequate exercise difficult, calisthenics of a mild type are supervised by our physical therapist, and we bring in an exercycle at times. Although it may seem strange, these patients have all enjoyed their stay and many have volunteered for another period if needed. None has quit because of claustrophobia, but one had to be sent to the surgical ward because of an incarcerated hernia. This latter instance was the only interruption in the experimental periods.

Thus far, 10 patients with rheumatoid arthritis, 2 with osteoarthritis, and 4 with pulmonary emphysema habe been studied in the Climatron. All had been chosen because they were good "weather prophets," complaining of increased symptoms before or during various weather changes. They

were paired by sex, and as closely as possible by age level, type of disease, and intelligence. All kept independant diaries, noting body weight 4 times daily, amount of fluid intake and output, periodic mouth temperature, and the time and character of any change they noted in the environment or in the symptoms and signs of the disease.

At least once daily each patient was evaluated by an experienced research physician for changes in strength, degree of joint tenderness, time to get up from a chair and walk a standard distance, blood pressure, pulse, and respiration. Pulmonary function tests were substituted in the emphysema cases. Other special tests were added in specific experiments. Each day's diary sheets were then checked and collected, the data charts of the climatic factors for the previous 24 hours also collected, and the data entered on the large running chart of the experiment.

I have purposely taken a long time getting around to results, because this is a long-term study and the results are not yet conclusive. The 12 arthritics and 4 emphysema cases are too small a number for conclusive data, and the possible permutations and combinations of our parameters of stydy have only been sampled in the first 10 months of what will be at least a 5-year study.

No results on the emphysema cases will be presented here, even though they have been somewhat similar to the arthritic results so far. This phase of the study is under the direction of Robert L. Mayock and the Pulmonary Function Laboratory of our institution.

Preliminary results in the 10 patients with rheumatoid arthritis and 2 with osteoarthritis, covering 296 patient-days of observation, have shown that a constant climate in the chamber nullifies completely any effect of weather change externally as observed by the patients through their window. Remarks in the diaries such as "It's raining today, and I didn't feel it coming" have been common. All periods of observation began with at least 5 days of constant climate for baseline observations and psychological and physical adjustment to the conditions of the new environment.

With all other parameters constant, falling or rising barometric pressure between 28.5 and 31,5 in. of mercury have shown no consistent effect upon arthritic symptoms or signs. There has been an interesting effect of these variations of fluid balance, however. Variation in the balance between intake and output of fluid, and in daily weights seem to indicate that there may be some fluid retention with increasing barometric pressure, and increased fluid loss with falling pressure. This is not conclusive as yet, and requires continued observation. It does, however, seem exactly opposite to what most of us had believed would happen.

Positive or negative air ionization, in concentrations as high as 50,000 ions/ml. of air has as yet produced no detectable effect on arthritic symtoms or signs in any of the 12 patients. The patients were completely unaware of any environmental change with even marked variations of ionization, or even of atmospheric pressure unless that latter was changed suddenly.

Whereas all patients could easily detect marked variations in temperature, humidity, or rate of air movement in the degree of their general comfort, none of these latter parameters has so far produced any consistent or significant effect on arthritic symptoms and signs when varied *singly*. Chilling, produced by various changes, did make some of the patients note increased stiffness and discomfort, however. Further observations on this point are necessary before conclusions can be drawn, since the data are so far rather inconsistent.

Strangely, one of the most significant observations so far has been the consistent increase in arthritic stiffness and soreness which appeared the day following exercise instruction by the physical therapist. She was instructed to visit the chamber without our knowledge on days of her choice, and to omit others. By both diaries and our examinations we could detect each visit, with the attendant increase in physical activity by the patients, on the day following. It seems certain that unaccustomed physical activity, with climatic factors constant, invariably increases arthritic discomfort. We wish we could find a factor or combination of climatic factors that would as consistently reproduce this worsening of symptoms, so that we could study in more detail the "weather effect" on arthritis and other human diseases.

Our results so far, using only single factor variations, have been negative. We must increase our data, and include many combinations and degrees of variation of factors to discover a detrimental weather combination. Our studies will continue.

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DESIGN OF CLIMATE-CONTROL CHAMBER*

John Everetts, Jr.

Charles S. Leopold, Inc., Philadelphia, Pa.

The climate-control chamber located in the Rehabilitation Building of the Hospital of the University of Pennsylvania was designed to reproduce atmospheric conditions, except for solar radiation, within the limits prescribed by Joseph L. Hollander.

The conditions to be maintained are contained in an area on a psychrometric chart bounded by a dry bulb range from 65° F. to 120° F., relative humidity of 80 per cent from 65 dry bulb to 80 dry bulb, a maximum wet bulb of 75° F. to 120° F. dry bulb, and a minimum dew point of 40° F.

In addition, barometric pressure can be varied from 28 to 32 in. Hg., and positive or negative ionization can be provided up to 60,000 counts per cc.

The basic chamber is a self contained reinforced concrete structure built within the main building structure. The combination bedroom living room is approximately 18 feet wide by 15 feet deep with a hung ceiling 8 feet above the floor. Adjacent to this room and contained within the pressure boundaries is a complete bathroom. A double door air lock is provided for access and 1 double glass window is included in the north wall.

The air-handling system consists of a pressure blower to recirculate the air; a double turboblower to provide fresh air, exhaust air, and barometric pressure control; filtration equipment; cooling and heating coils; an air saturator; automatic controls; acoustic chambers, supply and return duct work, and perforated ceiling air distribution.

Fresh air is brought into the system from the conditioned area of the main building through one of the turboblowers and discharged into the intake side of the recirculating pressure blower where it is mixed with the return air from the chamber. The air is then discharged into the conditioning equipment where it passes through a roughing filter, an absolute filter where particulate matter is removed down to 0.30 μ and then through a charcoal filter to remove odors. Each filter is provided with a filter gauge to determine when cleaning is required.

From the filters, the air passes through a cooling coil through which a glycol solution is circulated and which cools the air to the required dew-point temperature. The air then passes through a saturator and then through reheat coils where it is heated to the desired dry bulb before being distributed into the chamber. Perforated ceiling distribution is used to avoid drafts, particularly where high humidity conditions are to be maintained, which requires a higher rate of circulation than could be

*This paper, illustrated with slides, was the second of two papers presented at a meeting of the Division on November 28, 1961.

accomplished through formal grilles or ceiling outlets. Remote speed control is provided on the recirculating fan to modulate the air quantity within the limits of the required conditions. All apparatus and ductwork are built to operate at positive or negative pressures up to 2 lbs. gauge. Acoustic chambers are provided to prevent apparatus noise from disturbing the occupants in the climate chamber.

The plumbing for the bathroom, which is normally quite simple, became rather complex because of the differences in pressure between the control chamber and building sewage system. The maximum pressure differential required traps five feet deep that could not be flushed out under ordinary conditions with standard fixtures. It was therefore necessary to provide a sewage disposal sump with duplex pumps and specially designed motorized valves in the soil line to the sump, from the pump discharge to the main sewer, and the vent lines. The vent line itself had to be terminated in the bathroom to equalize the pressure on the drainage system during normal operations and necessitated an activated carbon filter in the line to prevent odors from getting back to the control chamber. A system of warning lights also had to be provided to warn the occupants not to use the bathroom so long as the yellow or red lights were on. The yellow light indicated that the sump tank was about ready to be emptied and the red light indicated the pumps were running and the soil line valve was shut. As the yellow and red lights were only on for two or three minutes at a time, and for only two or three times a day, this was not a serious problem once the occupants were educated to the signals. The biggest problem was to get a special ruling passed by the City Plumbing Inspection Department to allow valves in the lines, which was a direct violation of the existing codes and ordinances. The city of Philadelphia authorities were most cooperative, and it was not long before we had the necessary waivers so we could proceed with the work.

The refrigeration system was divided into two separate and complete circuits to assure continuity of operation in case of malfunction or breakdown of any one of the many components. The refrigeration load under the maximum load requirements was 13 tons. Each system has a capacity of 6.8 tons that will take care of approximately 70 per cent of the area shown on the psychrometric chart. The second system needs only to be used for supplementary refrigeration required for high duty loads or as a spare in case the first system becomes inoperative. A manual throw-over switch is provided to change the lead system from the first to the second, which is usually done every couple of weeks.

Each system consists of a belt-driven compressor with a 10-horsepower motor; oil separator, accumulator, and suction-liquid heat exchanger; necessary relief valves, a filter dryer, moisture and flow indicators; receiver and gravity air-cooled condenser. This type of condenser was selected, first, because water was not desired and, second, because there was no noise to disturb patients in the adjacent general

hospital building. The condenser circuit was provided with an automatic head-pressure control to compensate for outside temperature changes to maintain a constant head pressure on the system.

A common evaporator of the shell and tube type was provided with two separate refrigerant circuits, one circuit for each system. The evaporator was designed to cool a glycol solution from 40 F. to 33 F. with 26 F. refrigerant temperature. Each system is completely automatic throughout the entire range of capacities and requires only periodic maintenance instead of constant supervision.

The compressors were selected to operate at slow speed to minimize wear and reduce maintenance. The operational duty required for this system keeps the compressors in operation approximately 7000 hours a year, which is roughly 6 years of operation for the average compressor used in air-conditioning for comfort. As most of the test runs in the control chamber are for periods of 4 weeks of continuous operation, the continuity of operation is most important; therefore the proper selection of equipment for both duty and operation is essential.

The automatic-control system was designed to control four conditions: (1) dry-bulb temperature, (2) dew-point temperature, (3) glycol temperature, and (4) room pressure. All instruments are pneumatically operated and are of heavy-duty industrial design. Recorder-controllers are provided for dry-bulb, dew-point, glycol temperature, and room pressure, The dry-bulb, dew-point, and pressure controllers are also provided with individual automatic cam reset instruments to reset the controllers for any predetermined constant condition or any cyclical variation in conditions over short, or long periods of time. The glycol temperature is maintained constant at all times in a storage tank, from which it is drawn into the system as needed.

The dry-bulb temperature is controlled through the recorder controller by a thermostatic bulb located in the return air duct from the control chamber, which indicates the maintained dry bulb in the chamber. The temperature is controlled through the operation of a modulating steam valve raising or lowering the water temperature of hot water supplied to the reheat coils. As the air entering the reheaters is always at the dew-point temperature, and therefore always lower than room temperature, heat is always required to provide the desired dry bulb.

The dew-point temperature is controlled through a recorder controller by a thermostatic bulb located directly after the capillary washer or saturator. In a rise in control temperature, a three-way valve permits more cold glycol from storage tank to the cooling coils. On a drop in control temperature, less glycol is taken from the storage tank and more is recirculated to raise the cooling-coil temperature. If a high dew point is required, the three-way valve shuts off the flow of cold glycol from the storage tank and opens a steam valve to heat the water in the saturator, thereby increasing the dew point to the room.

The glycol temperature is controlled by a thermostat located in the storage tank to maintain a constant storage temperature. The refrigeration system cycle is demanded by the draw on the storage tank and is not connected in any way with the controlled conditions in the climate chamber. In this way a more accurate control of conditions can be maintained, particularly where cyclical changes are required. The control system was designed to control dry bulb and dew point within one half degree plus or minus the set point or a maximum of 1-degree variation. This would result in a total change of relative humidity of about 3 per cent and a change in absolute humidity of about 3 to 4 gr. per pound of dry air. These changes are insignificant for the test purposes of the control chamber. Actual service experience has indicated even closer control than design requirements.

Room pressure is controlled through a recorder-controller operated by a pressure transmitter, which receives its pressure signal from a pressure tip located in the return air duct from the control chamber. For a given control chamber pressure, if the barometric pressure of the atmosphere is lower, the controller will modulate the exhaust air valve to a partially closed position to permit the turboblower to build up the required pressure through the introduction of outside air. If the outside pressure is higher than the required control chamber pressure then the controller will throttle the fresh air valve and open the exhaust air valve so that the turboblower will create a partial vacuum to maintain the control chamber pressure lower than the atmospheric pressure. The degree of control as recorded is within one tenth of an inch of mercury, which again, does not significantly affect the test results.

The ionization equipment consists of an ion generator, either positive or negative; ion discharge terminals consisting of a one-half in. brass ball with three number 10 sewing needles set at 120° centers and at a 30° downward angle; a Philco ion counter and a Leeds and Northrup recorder.

The ion discharge terminals and the counter are located in the control chamber, while the generator and recorder are on the control panel outside the chamber. The generator can be set at any predetermined constant rate. Cyclic control is not provided.

Additional recorders are provided to indicate and record the total air supply to the chamber and the quantity of fresh air being introduced into the system. Automatic alarms and safety controls are provided for normal protection.

There were no special problems with the electrical system. A central panel with circuit breakers for each motor is provided. In general the system was designed basically for accurate control within the prescribed limits, with heavy duty equipment to assure long life, and duplicate equipment, where necessary, to provide continuity of service.

CONFERENCES HELD

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

RHYTHMIC FUNCTIONS IN THE LIVING SYSTEM

Wednesday, November 8,
Thursday, November 9,
Friday, November 10, and
Saturday, November 11, 1961

Conference Chairman: William Wolf
New York University, New York, N. Y.

WEDNESDAY, NOVEMBER 8, 1961

BASIC CONSIDERATIONS IN THE STUDY OF RHYTHMS

Session Chairman: Hans Kalmus
University College of London, London, England

9:00 A.M. –
Greetings from the Academy - Charles Noback, Chairman, Section of Biological and Medical Sciences, The New York Academy of Sciences, New York, N.Y.; College of Physicians and Surgeons, Columbia University, New York, N. Y.

Introductory Remarks - William Wolf, New York University, New York, N. Y.

"General Properties of Biologic Rhythms" - Arne Sollberger, Karolinska Institute, Stockholm, Sweden

"Temporal Parameters in Biology" - Franz Halberg, University of Minnesota, Minneapolis, Minn.

"Extrinsic Rhythmicality: A Reference Frame for Biologic Rhythms Under So-called Constant Conditions" - Frank A. Brown, Northwestern University, Evanston, Ill.

"Basimetric Approach (Law of Initial Value, LIV) to Biologic Rhythms" - Joseph Wilder, New York Medical College, New York, N. Y.

Session Chairman: Lawrence Stark
Massachusetts Institute of Technology, Cambridge, Mass.

2:00 P.M. –
"Biologic Rhythms and the Interaction of Frequencies" - Norbert Wiener, Massachusetts Institute of Technology, Cambridge, Mass.

"Adaptive Analog Models for Biologic Rhythms" - Otto Schmitt, University of Minnesota, Minneapolis, Minn.

"Basic Mathematical and Statistical Considerations in the Study of Rhythms and Near-Rhythms" - L. Hurwicz, University of Minnesota, Minneapolis, Minn.

"Circadian Systems" - Colin S. Pittendrigh, Princeton University, Princeton, N. J.

"Biologic Rhythms Due to the Biocybernetic Law of Unidirectional Rate Sensitivity" - Manfred Clynes, Rockland State Hospital, Orangeburg, N. Y.

"Telemetering Techniques for Periodicity Studies" - Guy Miles, University of Minnesota, Minneapolis, Minn.

THURSDAY, NOVEMBER 9, 1961

RHYTHMIC PROCESSES IN PLANTS AND ANIMALS

Session Chairman: Frank A. Brown
Northwestern University, Evanston, Ill.

9:00 A.M. –

"Ecological Influences in the Autonomous 24-Hour Rhythm in Plants" - Fritz Went, Missouri Botanical Gardens, St. Louis, Mo.

"Mechanism in Circadian Rhythms in Plants and Animals: Functional and Pathologic Changes Resulting from Rhythm Abnormalities" - Erwin Buenning, Botanical Institute, University of Tuebingen, Tuebingen, Germany

"Biochemical Aspects: Relation of Enzyme and Substrate Fluctuations to Persistent Circadian Rhythms" - J. W. Hastings, University of Illinois, Urbana, Ill.

"The Role of the Endocrine System in the Control of Certain Biological Rhythms in Birds" - A. V. Nalbandov, University of Illinois, Urbana, Ill.

"The Multiple Nervous and Hormonal Control of Circadian Rhythms in Insects" - Janet Harker, Zoological Laboratory, Cambridge, England

"Circadian Melanophore Rhythms of the Fiddler Crab: Interaction Between Animals" - Grover C. Stephens, University of Minnesota, Minneapolis, Minn.

RHYTHMIC EVENTS IN HUMAN PHYSIOLOGIC PROCESSES

Session Chairman: Franz Halberg
University of Minnesota School of Medicine
Minneapolis, Minn.

2:00 P.M. –

"The Adrenal Cycle" - Erhard Haus, University of Minnesota School of Medicine, Minneapolis, Minn.

"Relationship of Circadian Variations in Electrolyte Excretion to that of Adrenal Steroid Excretion" - Frederick C. Bartter, National Heart Institute, Public Health Service, Bethesda, Md.

"Periodicity of Leukocytes in Normal and Adrenalectomized Animals" - Thomas F. Daugherty, University of Utah College of Medicine, Salt Lake City, Utah

"Daily Rhythm in Leukocytes, Blood Pressure, Pulse Rate, and Temperature During Pregnancy: Characteristics of Certain Rhythms in Menstruation and Lactation" - Jiri Malek, Charles University, Prague, Czechoslovakia

"Rhythmic Circulatory Changes in the Peripheral Vasculature" - Kichinosuke Tatai, Institute of Public Health, Tokyo, Japan

FRIDAY, NOVEMBER 10, 1961

Session Chairman: Erwin Buenning
Botanical Institute, Tuebingen, Germany

9:00 A.M. –

"Periodic Phenomena in Genetical Systems" - Hans Kalmus, University College of London, London, England

"The Temperature Method for Studying Physiological Rhythms" - Nathaniel Kleitman, Santa Monica, Calif.

"Pupillary Rhythms and Hippus Fluctuations" - Lawrence Stark, Massachusetts Institute of Technology, Cambridge, Mass.

"Day-Night Cycling in Global Atmospheric Flight, Space Flight, and Other Global Bodies" - Hubertus Strughold, Brooks Air Force Base, Medical Research Center, San Antonio, Texas

"Periodic Desynchronization in Humans Under Outer Space Conditions" - George T. Hauty, Civil Aero-Medical Research Institute, Oklahoma City, Okla.

"The Effect of Latitude on Cycles" - Leonard W. Wing, Ann Arbor, Mich.

THE LAW OF INITIAL VALUE (LIV).

Session Chairman: Joseph Wilder
New York Medical College, New York, N. Y.

2:00 P.M. –

"The Principle of Relaxation Oscillations as a Special Instance of the LIV in Cybernetic Functions" - H. Selbach, Free University of West Berlin, Charlottenburg, Germany

"Automic Functions and the LIV" - W. Bridger and J. Block, Albert Einstein College of Medicine, Bronx, N. Y.

"Final Levels as Related to the LIV" - D. Oken and H. Heath, Michael Reese Hospital and Medical Center, Chicago, Ill.

"LIV in Longitudinal Study of Autonomic Constitution: 4-Year Reproducibility of Intrastressor 30-Minute Stereotype" - J. I. Lacey, Fels Research Institute, Yellow Springs, Ohio

"Gastric Secretion and the LIV" - E. Brandt, University of Oklahoma School of Medicine, Oklahoma City, Okla.

"Urinary Secretion and Blood Sugar in Relation to the LIV" - A Karyokopoulos, University of Oklahoma, Oklahoma City, Okla.

SATURDAY, NOVEMBER 11, 1961

RHYTHMS IN CLINICAL MEDICINE, PSYCHIATRY, AND PSYCHOLOGY

Session Chairman: Arne Sollberger
Karolinska Institute, Stockholm, Sweden

9:00 A.M. –

"Effect of Basal Levels on Circulatory Responses Induced by CNS Stimulation" - H. F. Scherrer, University of British Columbia, Vancouver, B. C., Canada

"Fundamental Concepts Underlying the Metabolic Periodicity in Diabetes" - Jakob Moellerstroem, Swedish Diabetic Foundation, Stockholm, Sweden

"Microfilaria Infestation as an Instance of Periodic Phenomena Seen in Host-Parasite Relationships" - F. Hawking, National Institute for Medical Research, London, England

"Periodic Phenomena in Human Reproduction: The Circadian Cycle of the Onset of Labor and Delivery in Man" - Irwin H. Kaiser, University of Utah College of Medicine, Salt Lake City, Utah

"Periodicity in Urinary Excretion in Healthy and Nephropathic Individuals" - Werner Menzel, Sieveking Hospital, Hamburg, Germany

Session Chairman: Peter Suckling
State University of New York, Downstate Medical Center
Brooklyn, N. Y.

2:00 P.M. –

"Observations on Periodic and Nonperiodic Processes During Human Sleep" - Arthur Shapiro, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

"Manic Depressive Rhythm: Its Experimental Modification and the Nature of the Self Structure" - George G. Haydu, Creedmoor State Hospital, Jamaica, N. Y.

"Measurement and Applicability of Periodic Changes in the Electromagnetic Field of Healthy, Mentally, and Physically Diseased Individuals" - Leonard J. Ravitz, Norfolk, Va.

"Time: Its Breadth and Depth in Biologic Rhythms" - John J. Grebe, Nuclear and Basic Research Institute, Dow Chemical Company, Midland, Mich.

PANEL DISCUSSION - Frank A. Brown, Franz Halberg, Arne Sollberger, Peter Suckling, and Joseph Wilder.

"Summary of Conference" - Maurice B. Visscher, University of Minnesota Medical School, Minneapolis, Minn.

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

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Vice-Chairman

DIVISION OF INSTRUMENTATION AND

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

APPLICATIONS AND METHODS OF COUNTING AND SIZING IN MEDICINE AND BIOLOGY

November 29, 1961

Conference Chairman: Walter E. Tolles
Airborne Instruments Laboratory
Deer Park, Long Island, N. Y.

Wednesday, November 29, 1961

Session Chairman: George W. Brecher
Clinical Center, National Institutes of Health,
Bethesda, Md.

9:00 A.M. -
Greetings from the Academy - Andres Ferrari, Chairman, Division of Instrumentation, The New York Academy of Sciences, New York, N. Y.; Technicon Instruments Corporation, Chauncey, N. Y.

"An Automatic Counter for Blood Cells" - Peter L. Frommer, Cincinnati General Hospital, Cincinnati, Ohio

"Size Distribution of Erythrocytes" - George W. Brecher and George Z. Williams, Clinical Center, National Institutes of Health, Bethesda, Md.

"Automatic Counting of Platelets" - Eugene P. Cronkite and Clyde R. Sipe, Brookhaven National Laboratory, Upton, Long Island, N. Y.

"Enumeration of Spermatoza" - S. J. Segal and K. A. Laurence, The Rockefeller Institute, New York, N. Y.

"Counting and Sizing of Unicellular Marine Organisms" - J. W. Hastings, University of Illinois, Urbana, Ill., and Beatrice M. Sweeney, Yale University, New Haven, Conn.

Session Chairman: Walter E. Tolles
Airborne Instruments Laboratory
Deer Park, Long Island, N. Y.

2:00 P.M. -
"The Partichrome Analyzer for the Detection and Enumeration of Bacteria" - Sol S. Nelson, Orvil E. A. Bolduan, U. S. Army Chemical Corps Biological Laboratories, Fort Detrick, Md., and Williams A. Shurcliff, Polaroid Corporation, Cambridge, Mass.

"A Particle-Size Analyzer for Aerosols" - Victor R. Mumma, U. S. Army Chemical Corps Biological Laboratories, Fort Detrick, Md., Albert L. Thomas, Jr. and Robert H. Collins III, Southern Research Institute, Birmingham, Ala.

"Counting of Neutons by Flying-Spot Microscope" - H. P. Mansberg, Airborne Instruments Laboratory, Deer Park, Long Island, N. Y.

"General Purpose Visual Input for a Computer" - Paul V. C. Hough, Brookhaven National Laboratory, Upton, Long Island, N. Y.

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

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DIVISION OF INSTRUMENTATION

Andres Ferrari
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Vice-Chairman

NEW MEMBERS

Elected November 28, 1961

LIFE MEMBERSHIP

Gerard, Robert, Ph.D., Psychology. Supervisory Research Psychologist, Veterans Administration Neuropsychiatric Hospital, Los Angeles, Calif.

ACTIVE MEMBERSHIP

Abul-Haj, Suleiman K., M.D., Biological and Medical Sciences. Chief of Anatomical Pathology, Walter Reed General Hospital, Washington, D.C.

Asboe-Hansen, Gustav, M.D., Dermatology. Professor, University of Copenhagen, Copenhagen, Denmark.

Barlow, R. Craig, M.D., Internal Medicine. Private Practice, Ann Arbor, Mich.

Beeber, Allan R. A., Ph.D., Consulting-Research & Development. President, Commercial Research & Development Corp., Elizabeth, N. J.

Beitsch, Nathan, LLB, Chemistry. Supervisory Chemist, Chemical Research & Development Laboratories, Army Chemical Center, Md.

Belcher, Jane C., Ph.D., Biology. Professor, Sweet Briar College, Sweet Briar, Va.

Bello, Phillip, Sc.D., Electrical Engineering. Senior Scientist, Adcom, Inc., Cambridge, Mass.

Bender, Harvey Alan, Ph.D., Genetics. Assistant Professor of Biology, University of California, Berkeley, Calif.

Bent, Henry A., Ph.D., Physical and Inorganic Chemistry. Associate Professor, University of Minnesota, Minneapolis, Minn.

Bergs, Victor V., Ph.D., Virology. Assistant Research Specialist, Rutgers University, New Brunswick, N. J.

Bergsagel, Daniel E., D.Phil., Internal Medicine. Associate Internist, University of Texas, M. D. Anderson Hospital, Houston, Texas.

Bikfalvi, Andreas, M.D., Surgery. Surgeon, University of Giessen Clinic, Giessen-Lahn, Germany.

Brinigar, William S., Ph.D., Biophysical Chemistry. Research Associate, Yale University, New Haven, Conn.

Brooks, Martin M., Pod.D., Podiatry. Private Practice, Rego Park, N. Y.

Brotzman, Lloyd E., A.A., Meteorology. Regional Administrative Officer, U.S. Weather Bureau, New York, N. Y.

Buc, George L., Ph.D., Applied Spectroscopy. Assistant to the President, Fisher Scientific Co., Pittsburgh, Pa.

Burgers, A. C. J., Ph.D., Ethnology, Marine Biology. Zoology Laboratorium, Utrecht, Holland.

Cederbaum, Israel, Ph.D., Theory of Electrical Networks. Visiting Professor, Department of Electrical Engineering, University of California, Berkeley, Calif.

Chachaty, Claude, E.S.C.M., Chimie Des Radiations. Laboratoire De Chimie Physique, Paris, France.

Chaddock, Richard E., Ph.D., Chemical Engineering. Director of Development, Hercules Powder Co., Wilmington, Del.

Cockerline, Alan Wesley, Ph.D., Cell Physiology. Assistant Professor, Department of Biology, Texas Woman's University, Denton, Texas.

Coddington, Nancy E., B.A., Cell Physiology. University Fellow, New York

University, New York, N. Y.

Costa, Giovanni, Ph.D., Cancer Research. Associate Cancer Research Internist, Roswell Park Memorial Institute, Buffalo, N. Y.

Daly, Lawrence H., Ph.D., Spectroscopy. Associate Professor, Chemistry Department, New York State University College, Albany, N. Y.

Danishefsky, Isidore, Ph.D., Biochemistry. Associate Professor in Biochemistry, New York Medical College, New York, N. Y.

Davis, G. R. F., Ph.D., Insect Physiology. Research Officer (Agriculture) Canadian Department of Agriculture, Saskatoon, Sask., Canada.

Delavan, John Pine, M.Arch., Nuclear Engineering. Associate and Project Manager, Voorhees Walker Smith Smith & Haines, New York, N. Y.

Echlin, Francis A., M.D., Neurophysiology and Neurological Surgery. Professor of Clinical Neurosurgery, New York University-Bellevue Medical Center, New York, N. Y.

Elliott, Alice, Ph.D., Parasitology. Professor of Biology, Hope College, Holland, Mich.

Emmerson, John L., Ph.D., Toxicology and Drug Metabolism. Sr. Pharmacologist, Lilly Research Laboratories, Indianapolis, Ind.

Fisher, Edwin R., M.D., Pathology. Professor, University of Pittsburgh, Pittsburgh, Pa.

Fleming, John G., M.D., Obstetrics and Gynecology. Assistant Professor, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Getmanov-von der Mosel, V., M.D., Gynecology and Obstetrics. Private Practice, Weehawken, N. J.

Gratz, Pauline, Ed.D., Physiology. Instructor, Columbia University, New York, N. Y.

Green, Frank O., Ph.D., Biochemistry. Professor of Chemistry, Wheaton College, Wheaton, Ill.

Harris, Basil, M.D., Clinical Teaching Methods. Director Health Service, Flower & Fifth Avenue Hospitals, New York, N. Y.

Heller, John R., M.D., Medical Research Administration. President, Memorial Sloan-Kettering Cancer Center, New York, N. Y.

Herter, Frederic P., M.D., Surgery. Assistant Professor of Surgery, Columbia University, New York, N. Y.

Horsey, Eleanor F., M.S., Chemistry. Assistant for Technical Administration, Diamond Ordnance Fuze Labs., Washington, D. C.

Howells, Cyril H. L., M.D., Pathology. Consultant, Royal Hospital, Wolverhampton, Staffs., England.

Iglesias, Rigoberto, M.D., Experimental Cancer Research. Director, Instituto de Medicina Experimental del Servicio Nacional de Salud, Santiago de Chile, Chile.

Igna, Eli J., M.D., Gynecology and Obstetrics. Associate Gynecologist and Obstetrician, Henry Ford Hospital, Detroit, Mich.

Jameson, A. Gregory, M.D., Hemodynamics. Director Cardiovascular Lab., Columbia Presbyterian Medical Center, New York, N. Y.

Jiménez-Porras, Jesús María, Ph.D., Biochemistry. Head of Biochemistry Department, University of Costa Rica, Costa Rica, C. A.

Lapointe, Marcel, M.D., Medicine. Private Practice, Hôtel-Dieu St.-Vallier, Chicoutimi, Que., Canada.

Levine, Norman David, M.D., Radiobiology. Senior Assistant Surgeon, USPHS, National Institutes of Health, Bethesda, Md.

Lifton, Robert Jay, M.D., Psychiatric Research. Associate Professor of Psychiatry, Yale University, New Haven, Conn.

Lindan, Olgierd, Ph.D., Human Metabolism. Assistant Professor Medicine,

Highland View Hospital, Cleveland, Ohio.

Loewe, Paul, M.D., Medical Radiology. Radiologist, Hospital de la Raza, Mexico, D. F., Mexico.

McCarroll, James Guthrie, M.D., Obstetrics and Gynecology. Visiting Consultant, University Hospital, Saskatoon, Sask., Canada.

McCreesh, Arthur H., Ph.D., Drug Screening. Pharmacologist, U.S. Army Chemical Center, Md.

McEwen, Donald Cameron, M.D., Medicine. Consultant, Regina General Hospital, Regina, Sask., Canada.

McGeary, George D., M.D., Pathology. Pathologist and Director of Laboratories, St. Charles Hospital, Bend, Ore.

Maccacaro, Giulio Alfredo, M.D., General Microbiology. Professor of Microbiology, University of Modena, Milano, Italy.

Mack, Ernest Wood, M.D., Neurosurgery. Private Practice, Reno, Nev.

Mackles, Leonard, B.S., Organic Chemistry. Chief Chemist, Schenley Research Institute, New York, N. Y.

Mansberger, Arlie R. Jr., M.D., Experimental Surgery. Associate Professor of Surgery, University of Maryland Hospital, Baltimore, Md.

Mereness, Dorothy, Ed.D., Psychiatric Nursing. Associate Professor of Education, New York University, New York, N. Y.

Michaels, Alan S., Sc.D., High Polymers. Professor, Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Mass.

Miller, Marvin D., B.S., Food Technology. Senior Food Technologist, American Sugar Refining Co., Brooklyn, N. Y.

Naficy, Kiarash, M.D., Infectious diseases. Research Fellow, Harvard Medical School, Boston, Mass.

Noble, Ernest P., Ph.D., Carbohydrate Metabolism. Senior Instructor in Biochemistry, Western Reserve University, Cleveland, Ohio.

Nwagbo, Uzoechina, M.D., General Surgery. Research Fellow, Harlem Hospital, New York, N. Y.

Nystrom, C. A., M.D., Obesity and Preventive Medicine. Cawker City Clinic, Cawker City, Kans.

Opik, Ernest Julius, Doctor Phil.Nat., Astronomy. Visiting Research Professor of Astrophysics, University of Maryland, College Park, Md.

Oppenheimer, Armand M., D.D.S., Anthropology. Associate Clinical Professor of Dentistry, Columbia University, New York, N. Y.

Pignataro, Anthony, S.C.D., Biology. Basic Science Department, Atlantic States Chiropractic Institute, New York, N. Y.

Prince, Herbert Norman, Ph.D., Chemotherapy. Senior Virologist, Hoffmann-La Roche, Inc., Nutley, N. J.

Raiser, Helmut, B.S., Pharmaceuticals. President, FBA Pharmaceuticals, Inc., New York, N. Y.

Ridlon, Seward A., Ph.D., Pharmacology. Senior Pharmacologist, Bristol Laboratories, Syracuse, N. Y.

Riss, Eric, Ph.D., Psychology. Chairman of the Board, Scientific Introduction Service Inc., New York, N. Y.

Runyon, Richard P., Ph.D., Psychology. Chairman, C. W. Post College, Greenvale, L. I., N. Y.

Sanders, H. D., M.Sc., Medical Sciences. Graduate Student, University of Manitoba, Winnipeg, Manitoba, Canada.

Santulli, Thomas V., M.D., Pediatric Surgery. Associate Professor of Clinical Surgery, Columbia University, New York, N. Y.

Schuel, Herbert, Ph.D., Cellular Physiology. United States Public Health Service Postdoctoral Fellow, Oak Ridge, Tenn.

Shacter, John, B.S., Separations Processes. Manager of Planning, Union Carbide Corp., New York, N. Y.

Simpson, George, M.D., Psychiatry. Senior Psychiatrist, Rockland State Hospital, Orangeburg, N. Y.

Smirk, Frederick Horace, Sir., D.Sc., Hypertension. Professor of Medicine, University of Otago, Dunedin, New Zealand.

Somerville, Edgar William, M.B., Orthopaedic Surgery. Consultant, Nuffield Orthopaedic Centre, Oxford, England.

Spirakis, Pericles, B.E.E., Electrical Engineering. Senior Member Technical Staff, Radio Corporation of America, New York, N. Y.

Sutton, Samuel, Ph.D., Experimental Psychopathology. Associate Research Scientist, Columbia University, New York, N. Y.

Symeonidis, Alexander, Ph.D., Cancer Research. Professor of Pathology, Aristotelian University, Thessaloniki, Greece.

Tebo, Julian D., Dr. Eng., Magnetics. Head, Technical Relations Department, Bell Telephone Labs., New York, N. Y.

Thormen, George S., Biorhythm. President, Biorhythm Computers, Inc., New York, N. Y.

Tilford, Charles H., Ph.D., Medicinal Chemistry. Director of Chemical Research, Warner-Lambert Research Institute, Morris Plains, N. J.

Tuppy, Hans, Ph.D., Biochemistry. Professor of Biochemistry, University of Vienna, Vienna, Austria.

Udall, Robert H., Ph.D., Animal Nutrition. Associate Professor of Animal Nutrition, Colorado State University, Fort Collins, Colo.

Usami, Shunichi, M.D., Climatic Physiology. Instructor of Physiology, Columbia University, NewYork, N. Y.

Villaverde, Manuel M., M.D., Endocrinology. Medical Advisor, International Division, Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.

von Grün, Leopold, Ph.D., Nuclear Physics. Research Physicist, Private Practice, Brooklyn, N. Y.

Walsh, John Breffni, MSEE., Radar Systems. Assistant Director, Columbia University, NewYork, N. Y.

Welter, Eugene S., M.D., Medicine. Private Practice, Aurora, Ill.

Werne, Benjamin, S.J.D., Medical Jurisprudence. Lecturer, Columbia University, New York, N. Y.

Whiteside, Robert S., Food Chemistry. President, Sterwin Chemicals, Inc., New York, N. Y.

Williams, Josephine J., Ph.D., Sociology. Research Associate, Columbia University, New York, N. Y.

Wilson, William Ralph, M.D., Cardiovascular Disease, Assistant Professor, University Hospital, Iowa City, Iowa.

Windsor, Emanuel, Ph.D., Biochemistry. Senior Biochemist, Riker Laboratories, Inc., Northridge, Calif.

Wood, John Archibald, M.D., Cardiopulmonary. Associate in Medicine, Columbia University, New York, N. Y.

Woolsey, Elwood Larkins, M.D., Obstetrics & Gynecology. Senior Consultant. Miner's Memorial Hospital Association, Harlan, Ky.

Wright, Edwin Thorpe, M.D., Biological and Medical Sciences. Chief, Dermatology Section, VA Center, Los Angeles, Calif.

Wüst, Georg, Ph.D., Oceanography. Emeritus Professor, University of Kiel, Kiel, Germany.

Yancey, Asa G., M.D., Biological and Medical Sciences. Chief of Surgery, Hughes Spalding Pavilion of Grady Memorial Hospital, Atlanta, Ga.

Young, Forrest, M.D., Surgery. Private Practice, Redlands, Calif.

STUDENT MEMBERSHIP

Laughlin, John L., B.S., Social Work and Psychotherapy. Graduate Student, New York School of Social Work, Columbia University, New York, N. Y.

Sankaranarayanan, Krishnaswami, M.A., Genetics. Predoctoral University Fellow, Teaching Assistant, Zoology Department, Columbia University, New York, N. Y.